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The effect of nutrition on the morphology of the wool follicle

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**THE EFFECT OF NUTRITION ON THE MORPHOLOGY OF THE WOOL
FOLLICLE**

Gail Moriarty

**A thesis submitted in partial fulfilment of the
requirements for the degree of Bachelor of Applied**

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Faculty of Science and Technology

USE OF THESIS

The Use of Thesis statement is not included in this version of the thesis.

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ABSTRACT

This project was part of a larger CSIRO experiment which was examining the factors involved in the development of tender wool. In order to understand the effect of nutritional variations on wool quality, changes in cellular activity and morphology within the wool follicle were examined.

Six sheep with a history of producing low staple strength wool (< 25 N/ktex) and six sheep with a history of producing high staple strength wool (> 30 N/ktex) were selected. The experiment ran for 18 weeks, for the first 10 weeks the sheep were fed a restricted ration to achieve a weight loss of approximately 175 g/day. From weeks 11 to 18, the sheep were fed an iso-energetic ration containing low or high protein. The sheep were weighed weekly and colchicine treated skin biopsies were collected at 0, 2, 10, 11, 12 and 18 weeks, embedded in paraffin and serial sectioned.

The work carried out for this Honours project involved assessing wool follicles from these sheep to determine the stage of the growth cycle, the mitotic rate and the area of the follicle bulb.

Although group differences were not evident between the different diets and staple strengths, this study demonstrated a high correlation between the number of mitotically active cells in the follicular bulb, the area of the follicular bulb, live-weight of the sheep, clean wool weight and fibre diameter ($r^2 = 84.5\%$, $p = 0.000$).

It was found that after ten weeks on restricted ration the live-weight, mitotic counts of follicular bulb cells and follicle bulb areas for all groups decreased from pre-treatment. When followed by a change of diet from the restricted ration to the iso-energetic ration for eight weeks the live-weights, mitotic counts and follicle bulb areas in all groups increased to be higher than pre-treatment.

This study found that restricted food intake resulted in decreased cell division in the follicle bulb which is concomitant with decreased follicle bulb area. This was accompanied by a decrease in wool production and a shift toward the telogen stage of the growth cycle. Follicles in telogen stage of the growth cycle were identified for the first time in the Merino sheep. This suggests that follicles are less active, that is, have less cell division on the restricted ration but that the mitotic activity rises again on a higher energy ration, irrespective of staple strength history or low or high protein ration.

DECLARATION

"I certify that this thesis does not incorporate without acknowledgment any material previously submitted for a degree or diploma in any institution of higher education; and that to the best of my knowledge and belief it does not contain any material previously published or written by another person except where due reference is made in the text."

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CHAPTER 1. INTRODUCTION

Low staple strength or tenderness in the Australian wool clip is a problem of economic importance estimated to be in excess of forty million dollars in Western Australia alone and one hundred million dollars Australia wide (Ralph 1986). Thompson and Curtis (1990) reported changes in wool production associated with seasonal fluctuations in pasture quality and quantity. Tender wool in Western Australia is associated with the autumn period of poor nutrient availability. The variation in wool quality becomes a problem when processing the wool and may result in the production of inferior quality textiles (Ainsworth 1989).

Staple strength is a measure of the strength of fibres in a staple (a cluster or group of fibres) and is recorded in Newtons/kilotex. Tender wool is wool with a staple strength less than twenty five Newtons/kilotex. Reduction in fibre diameter over a short period of time may also be associated with changes in the intrinsic strength of the staple and therefore is often associated with tenderness (Hansford and Kennedy 1988).

In order for the wool producer to produce wool of more consistent quality a greater understanding of the factors which influence wool growth is required. Wynn et al (1991) have highlighted the current poor understanding of the regulatory mechanisms in the wool follicle.

To understand the effect of nutritional variations on wool quality, changes in cellular activity within the follicle need to be examined. The present study was undertaken to determine whether changes in nutritional status influenced the dynamics (cell division and follicle area) of the wool follicles and therefore wool production.

This project was part of a larger CSIRO experiment which was examining the factors involved in the development of tender wool. In order to understand the effect of nutritional variations on wool quality, changes in cellular activity and morphology within the wool follicle were examined. A number of relationships were examined to gain an overall understanding of the dynamics of the wool follicle. These were: the rate of cell division and the size of the germinative region in the wool follicle in relation to staple strength of the wool fibre; weight gain and loss in sheep on different nutritional regimes; wool produced per unit area; and fibre diameter of wool fibres.

1.0. LITERATURE REVIEW

1.1. The Wool Follicle

Individual wool fibres arise as the result of cell division in the wool follicle. The development of wool follicles has been described by Wynn et al (1991) as the culmination of a complex set of interactions between the dermal and epidermal components of the skin. The cells in the bulb of the wool follicle are amongst the most proliferative in the body.

Sutton et al (1991) and Harmsworth and Day (1990) described the primary phase of wool follicle growth in the developing foetus as consisting of a trio of follicles, one centrally and two laterally placed. The first primary follicles have been found to appear ninety days before birth on sixty days after conception. The fibres from these follicles appeared above the skin's surface fifty days before birth.

Fraser and Short (1960) observed that the secondary follicles followed a similar pattern but each stage occurred fifteen to twenty days after the corresponding primary phase. The first formed secondaries appeared in groups of three to five in a semi-circular pattern some distance from each primary trio. Further development of the secondary follicles resulted in branches from the original secondaries and as many as eight secondary follicles may be

formed in this manner. Harmsworth and Day (1990) found that the later formed secondaries were initiated just before birth and matured rapidly during the second and third week after birth.

Wynn et al (1991) stated that the current evidence suggested that differences in the efficiency of wool production between genotypes were due to differences in follicle function. Follicle function has been found to be the result of complex interactions between the various constituent cell types and the surrounding dermal tissues. The regulatory mechanisms of the follicle are currently poorly understood.

1.2. Follicle Histology

Figure 1.1 shows a primary follicle with its wool fibre. The wool fibre originates in the follicle bulb and grows toward the epidermal surface where it penetrates outward through the skin. Primary follicles are characterised by the presence of a full complement of accessory structures; a sudoriferous and a sebaceous gland and an erector pili muscle. Secondary follicles usually have only sebaceous glands. (Nay 1975)

The follicle bulb makes up the inferior portion of the wool follicle. The wool follicle surrounds the dermal papilla.

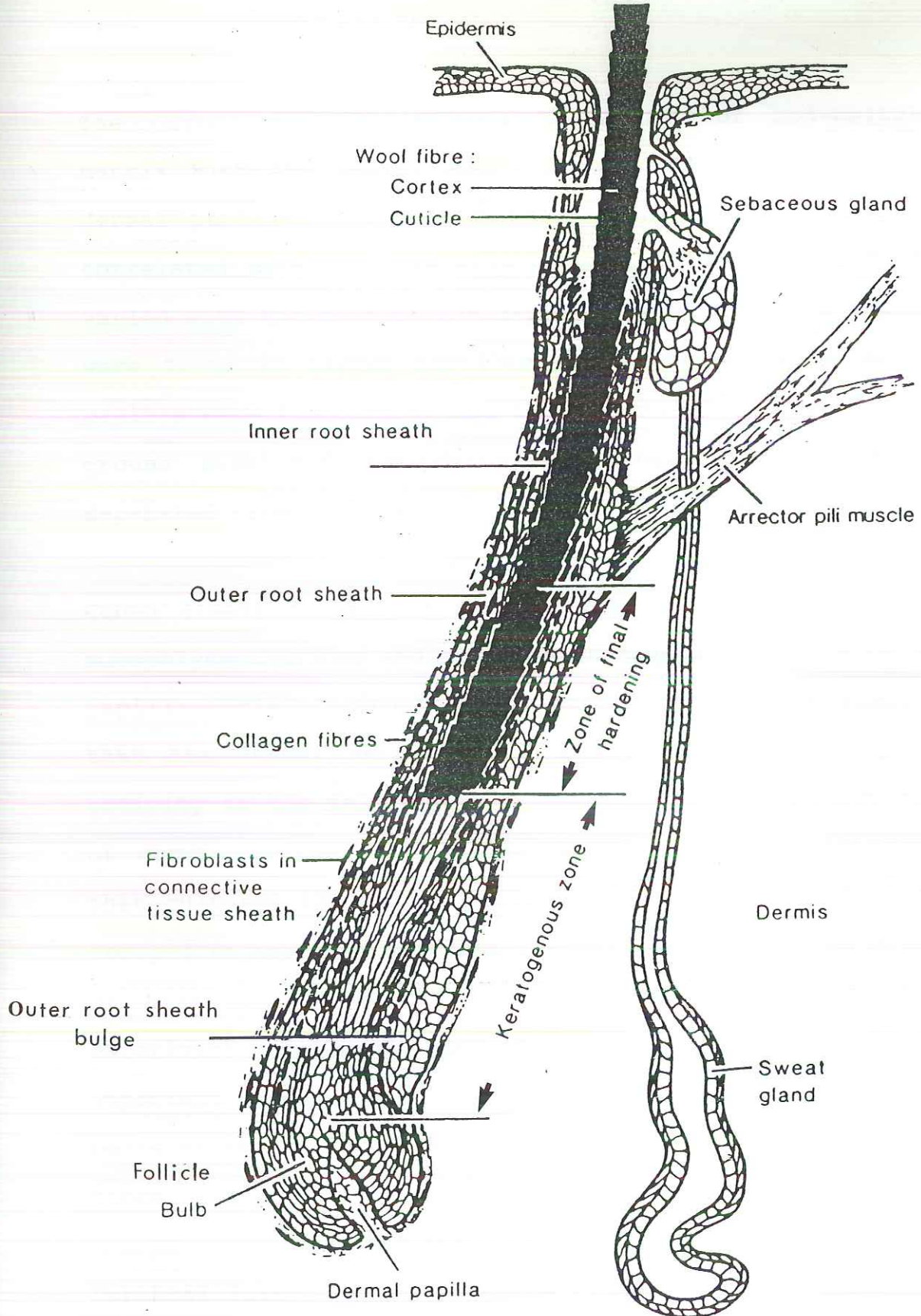


Figure 1.1. Diagram of primary follicle with a non-medullated wool fibre. (After Maddocks and Jackson 1988)

The dermal papilla is composed mostly of non-cellular matrix with few cells. Moore et al (1991) reported that dermal papilla size varied greatly and papilla size was correlated with follicle size. The vascularity of papillae varied with the size of the papilla, generally more vessels were found in larger papillae. Most of the cells of the papilla were fibroblasts and the non-cellular component was ground substance and collagen fibres. The papilla was separated from the cells of the bulb by a basal lamina.

Cohen (1965) found that the content of matrix (e.g. acid mucopolysaccharides and alkaline phosphatase) in the dermal papilla varied during the hair growth cycle. This suggests that the dermal matrix plays a role in determining the activity in the follicle bulb cells and therefore the rate of growth of the fibre. Reynolds and Jahoda (1991) found that cultured rat dermal papilla cells transplanted to rat footpad (a normally hairless tissue) resulted in the growth of hair fibres in the region of the transplant. This experiment confirmed that dermal papilla cells contain important growth factors which may induce mitosis in the cells of the follicle bulb and therefore growth of the hair fibre.

Parakkal (1967) stated that the cells of the follicle bulb were morphologically identical prior to differentiation. The particular cell into which a follicle bulb cell differentiated was correlated with its position within the

follicle. The cells adjacent to the dermal papilla gave rise to the medulla and cortex of the wool fibre while the cells at the base of the follicle bulb form the inner root sheath and cuticle (Figure 1.1.).

Epstein and Maibach (1967) used tritiated thymidine and autoradiography to determine the proliferation and movement of cells in the human hair follicle. Cells destined to form the inner root sheath moved into position more rapidly than did pre-cortical cells. The cells which moved to the outer root sheath divided several times during their passage up the hair follicle and this resulted in a bulge in the outer root sheath above the area of the dermal papilla.

1.3. Fibre Structure

Onions (1962) described the wool fibre as made up of several different types of cell. The outermost cells are hard, flattened, scale-like cells which make up the cuticle layer (Figure 1). The inner core is called the cortex and consists of rounded spindle shaped cells filled with many keratinised fibre bundles. In thicker wool fibres there may be a medulla which is formed from hollow rounded cells and found along the main axis of the fibre. Harmsworth and Day (1990) observed that medullated fibres had a cone shaped papilla, whereas non-medullated fibres had a dome-shaped papilla. Sims (1970) noted that human hairs consist of only cuticle and cortex cells.

Leeder (1991) described wool as a composite structure in which small pockets of protein material (the cuticle and cortical cells) are wrapped in a keratinised membrane and held together by a cell membrane complex. Whereas Powell (1991) described wool as a simpler fibre of keratins plus matrix proteins, the precise nature of which is still under investigation. Barnard (1962) reported that keratins were proteins with a high sulphur content. Rogers et al (1988) have found that the protein composition of wool varies and may be influenced by genetic origin, nutritional status and physiological state of the animal.

The process by which cells in the growing wool fibre harden and die is called keratinisation. This process starts approximately one third of the way up the follicle (Fig.1.1). In sheep keratinisation was not uniform across the cortex resulting in the characteristic crimping of Merino wools (Ryder 1973).

1.4. Wool Fibre Parameters

Staple strength is a measure of the strength of the wool fibres in a staple (a cluster or group of wool fibres) and is recorded in Newtons/kilotex (N/ktex). The force required to rupture the staple is measured in Newtons and kilotex is a measure of the clean staple linear density (1 tex = the weight in grams of 1 km of fibre). Maddocks and Jackson (1988) classified tender wool as wool with a staple

strength less than thirty N/ktex although the Australian Wool Corporation classifications are 25-30 (part tender), less than 25 (tender).

Harmsworth and Day (1990) reported differences in fibre diameter within a staple and along the length of a fibre. The variation in fibre diameter can be influenced by the age of the sheep, the part of the body on which the fibre has grown, nutritional state and stress factors. These stresses can be associated with poor nutrition, lambing, shearing and change of season or other factors that place demands upon the body reserves of the sheep resulting in the variation in fibre diameter (Wilkinson 1982).

Harmsworth and Day (1990) reported that a decrease in fibre diameter was correlated with decreased staple strength or tender wool. Bigham et al (1983) suggested that no more than forty per cent of the variation in staple strength could be accounted for by variations in fibre diameter. Therefore other factors may be involved in the production of tender wool. Orwin et al (1980) cited in Bigham et al (1983) suggested differences in protein composition as a factor contributing to the strength of wool fibres.

Hansford and Kennedy (1988) conducted a study to determine whether minimum fibre diameter or rate of change of fibre diameter was the most significant factor determining change in staple strength. They found that minimum fibre diameter

did not account for all of the variation and that lower staple strength was also associated with the rate of change in fibre diameter along the staple. Recent studies of staple strength have also shown that the shedding of fibres and the subsequent lack of continuity of fibres (due to the break in the staple caused by shedding) in the staple accounts for a significant proportion of the change in staple strength (Schlink et al 1992).

1.5. Growth Cycles of Hair and Wool Fibres

Ryder (1973) stated that in most mammals, after a follicle had completed development, the hair grew in cycles which consisted of periods of activity alternated between periods of rest. At the end of the resting phase the old hair is shed from the follicle. There are three main phases in the hair growth cycle; the active growth phase or anagen, a retrogressive phase catagen and a resting phase telogen. Rook (1970) observed that while the order of the stages did not vary the duration of the phases did. The length of the different phases can be measured readily in rats and mice as hair of these animals grows in waves and therefore follicles grow synchronously in a given area of the body.

Detailed descriptions of follicles, in mice, in the three stages of growth have been published (Parakkal 1970, 1990; Straile et al 1961). In contrast little is known about wool growth cycles in sheep although Ryder (1973) reported that

wild sheep have a simple annual cycle with a short active phase in summer followed by a long resting period in winter. In contrast Harmsworth and Day (1990) found Merino sheep to have continuous growth of wool throughout life under favourable conditions, with no regular cycling pattern of wool follicle growth. Ryder (1973) found that although plucking of hairs in mice stimulated the growth of a new hair, in sheep the follicle remained empty until regrowth in the surrounding follicles had taken place. This suggests that in sheep a complex mechanism involving more than one follicle is required to stimulate a new wool growth cycle.

1.5.1. Anagen

Flesch (1954) described the first sign of the beginning of anagen as an increase in mitotic activity accompanied by an elongation of the follicle. Parakkal (1990) noted that the lowest part of the follicle was an onion shaped bulb made up of undifferentiated matrix cells surrounding the dermal papilla. Ryder (1973) and Spearman (1973) stated that the follicle continues to enlarge as the new hair grows upwards. The matrix cells of the follicle bulb divide and differentiate to form the inner root sheath and the hair. The outermost ring of cells in the bulb form the outer root sheath which tapers from multi-layered in the upper part of the follicle to a single layer of cells at the lower end.

The lower part of the hair follicle is regenerated during anagen from a group of germ cells which surround the club during telogen. The germ cells undergo mitosis to form the new bulb of matrix cells which in turn give rise to the new hair shaft and inner root sheath (Parakkal 1970).

1.5.2. Catagen

Parakkal (1990) described catagen as a brief period during which the follicle is converted from the active growing phase to the resting phase. During catagen mitotic activity in the bulb cells of the follicle slows and finally ceases and the process leading to the formation of club hairs begins. The inner root sheath is lost and the lower part of the follicle shortens. The soft root of the hair keratinises into the brush end which is embedded in the surrounding germ cells which are transformed outer root sheath cells. These germ cells are the progenitors for the next hair.

1.5.3. Telogen

Telogen follicles have been found to differ structurally from anagen follicles. Ryder (1973) and Parakkal (1990) have described telogen follicles as one half to one third the length of anagen follicles. During telogen the dermal papilla was reduced to a ball of cells located directly below the fibre from the previous cycle. Between the brush

end of the fibre from the previous cycle and the isolated dermal papilla was the epithelial stalk made up of undifferentiated cells.

Rook (1970) made the following observations of sheep telogen follicles. They contained a well anchored "brush end" beneath which lay the germ cells and the dermal papilla. The brush end of the fibre was a hollow brush of keratinised cells at the lower end of the cortex. The brush or club end was usually retained until early anagen. Cells from the outer root sheath become the germ cells for the follicle bulb cells of the next hair growth cycle. These observations were made on primitive sheep breeds with annual shedding cycles but no descriptions of telogen follicles have been reported in the literature for Merino sheep.

Parakkal (1970) described telogen as a stable mature stage during which hair growth had ceased and the hairs were well anchored in the follicle. Parakkal (1990) observed that with appropriate stimulation the follicles could commence the growing phase, anagen. Conversely growing follicles in the presence of deleterious agents would go into telogen.

1.6. Factors Controlling the Cycle

Reynolds and Jahoda (1991) found that hair growth in rats occurs in skin grafts before nerves regenerate suggesting

that hair growth is not under nervous control. Intrinsic control of the cycle is indicated by the fact that hair in grafts shows the same pattern as the area from which the graft was taken and not that of the area to which it had been transferred. Lindner and Ferguson (1956) studied hormonal control of wool fibre growth in sheep with adrenocorticotrophic hormone (ACTH). Administration of ACTH resulted in follicles becoming inactive and shedding their fibres. Conversely thyroxine given during anagen in these experiments stimulated wool growth.

Ryder (1973) suggested that seasonal growth patterns, for example spring moulting, were triggered by changes in day length. One explanation for these annual cycles may be photoperiod stimulation of the anterior pituitary resulting in secretion of ACTH. In more recent studies, it has been proposed that prolactin is indicated in the seasonal moulting pattern of primitive sheep, Curlewis (1992) suggested that selective breeding for continuous fleece growth in domesticated sheep has led to the hair follicle becoming unresponsive to seasonal changes in hormone levels.

1.7. Nutrition and Wool Growth

Lyne (1964) and Harmsworth and Day (1990) reported that the number of follicles in an individual animal was determined

genetically but with diet having an influence on the rate of secondary follicle development. The last few months before birth and for a short period after birth are the critical times for secondary follicle development in Merino sheep. Lyne (1964) found that a few months after birth lambs on restricted rations had reduced fibre output but no permanent depletion of the follicle population. Fitzgerald et al (1984) found that varying the diet of ewes in mid-pregnancy resulted in significant differences (20-37 N/ktex $p < 0.001$) in staple strength of the resultant lambs.

Butcher et al (1984) found that staple strength of wool was decreased by 11-25 N/ktex between seasons in the same sheep where one season's conditions were more favourable than the next. In another study by the same authors it was observed that sheep with initial high staple strength suffered a greater decrease in staple strength (11 N/ktex reduction) than sheep with initial low staple strength (3 N/ktex reduction) following seasonal change resulting in poorer pasture quality. This would suggest that sheep with high staple strength wool are more sensitive to changes in nutritional state.

Both carbohydrates and proteins have important roles in wool production. Glycogen, which is stored in the follicles, is utilised during cell division and carbohydrates are also required for the synthesis of amino acids into proteins. Hocking Edwards and Hynd (1992) found

that strongwool Merinos have a greater skin uptake of glucose than finewool Merinos, indicating a higher energy requirement to maintain the high volume of germinative tissue associated with greater wool production.

Marsten (1955) found that amino acids were critical for fibre production particularly the sulphur containing amino acids (methionine and cysteine) which are in high concentrations in the wool fibre. Mier and Cotton (1976) and Reis (1991) also indicated the importance of the ratio of amino acids. Diets containing all the essential amino acids but with unbalanced ratios resulted in poor fibre growth.

Marsten (1955) suggested that amino acid concentration in the tissue fluids surrounding the wool follicles influenced wool growth. The concentration was determined initially by quality and quantity of protein ingested in feed and additionally by the amino acids made available by the bacterial breakdown of protein in the rumen. The amount that is made available to serve the follicles for wool production is determined by poorly understood metabolic processes that alter with the nutritional status of the animal.

Marsten (1955) found that plants have the greatest protein content when young, succulent and usually wet. When the fodder plants dried off the protein content was lower and

less easily assimilated. Fluctuations of over 400% in the rate of wool production were recorded between the lush feeding period of spring and the lean times of late autumn in Western Australia. Revell et al (1990) suggested that wool production began to decrease as soon as thirty days after pasture wilted.

Hynd (1989) reported an increase in wool production by sheep on a high protein diet. The high protein diet altered the length/diameter ratio, with the length of fibre increasing more than the diameter. The increase in wool production was accompanied by a thirty per cent increase in the germinative volume and a thirty five per cent increase in the number of cells in the bulb entering metaphase.

Moore et al (1982) observed an increase in wool production in sheep fed a diet equal in protein but consisting of four hundred grams per day more bulk than the control group. The sheep on the higher ration produced 190 mg clean wool per patch per day compared to 97 mg clean wool per patch per day on the lower ration. This result suggested that nutrients other than protein are critical for wool production and excessive protein may not be effectively utilised by the sheep.

The supply of minerals in the diet has been shown to affect wool growth. Reis (1991) stated that while many minerals affected wool growth, only the trace elements copper and

zinc were directly required in the process of wool growth. Copper deficiency reduced the amount of wool grown and zinc deficiency has been found to cause the cessation of fibre growth.

In humans extensive research has been carried out on subjects suffering from the deficiency diseases marasmus and kwashiorkor. Marliss (1985) described kwashiorkor as a condition resulting from inadequate protein intake and marasmus as a condition resulting from levels of protein and energy below that required for normal metabolic functioning of the body.

Bradfield and Bailey (1967) reported the following symptoms in children suffering from marasmus; a decrease in the hair shaft diameter, decreased serum albumin values, decreased strength of hair and a shift in the normal anagen:telogen ratio of eighty-twenty to a condition where few anagen follicles and approximately forty six per cent telogen follicles were found. Children suffering from kwashiorkor were found to have the following symptoms; atrophy of the hair follicle bulb, loss of outer root sheath, loss of inner root sheath and lowered hair cystine levels. These findings indicate the importance of the role of protein in hair production in humans.

1.8. Effect of Adrenal Hormones on Wool Growth

1.8. Effect of Adrenal Hormones on Wool Growth

The glucocorticoids; cortisol (hydrocortisone), corticosterone and cortisone are all hormones secreted by the adrenal cortex. Chapman and Bassett (1970) have shown that the glucocorticoids have an inhibitory effect on hair growth. In sheep, administration of cortisol to give a plasma cortisol concentration of two micrograms per decilitre, resulted in a decrease in fibre diameter. Wool growth was completely suppressed in sheep with plasma cortisol concentrations of three to five micrograms per decilitre. It was further observed that sheep could tolerate a slight rise in plasma cortisol, approximately one microgram per decilitre, when they were able to increase their food intake. Sheep on restricted rations were adversely affected by small increases in cortisol level. Observation of the fibres taken from sheep which had been given cortisol showed that the fibre was shed, indicating that the wool follicle had entered into catagen. Descriptions of the follicles were consistent with descriptions of follicles in telogen stage of the growth cycle.

The adrenal cortex has been shown to increase in activity during disease, pregnancy and as a result of environmental stress. Lindner and Ferguson (1956) suggested that an increase in adrenocortical secretion resulted in the production of tender wool.

1.8. Techniques for Estimating Wool Growth

Growth of the wool fibre is determined both by mitotic rate, growth and migration of cells in the proliferative area of the follicular bulb and the total cell population in this area. (Wilson and Short 1979; Chapman et al 1980; Marsten 1955)

Pioneering work in mitotic activity in wool follicle bulbs was carried out by Schinkel during the early nineteen sixties. Schinkel (1961) used intravenous doses of colchicine to arrest mitosis and concluded that measures of mitotic figures were useful in determining the proliferating cells of the wool follicle bulb and subsequent wool fibre growth. Colchicine is a strathmokinetic agent which arrests cells in metaphase by interrupting spindle formation. Schinkel (1962) concluded that mitotic rate as well as size of cells in the follicle bulb influenced wool growth. The use of intravenous doses of colchicine are often detrimental or even fatal to experimental animals.

To overcome the latter problem, Fraser (1962) and Hynd et al (1986) used intradermal doses of colchicine and selective staining to quantify metaphase-blocked nuclei in the follicular bulb and examined the relationship between follicle activity and fibre production with nutritional changes. Using this method the effect of the colchicine is

confined to an area of approximately five centimetres radius from the injection site allowing for repetition of counts in the same animal. More recently another method for measuring rate of cell division in follicles using 5-bromo-2'-deoxyuridine (BrdU) has been described, which labels cells in the S-phase of the cell cycle. (Hynd and Everett 1990; Holle and Harris 1992)

Hynd and Everett (1990) compared the colchicine and BrdU methods and obtained higher rates of cell division with the BrdU method. Using this data they suggest that the colchicine method underestimates cell birth rate in wool follicles. However the BrdU method calculates the number of cells in the S-phase of the cell cycle while the colchicine method calculates those in metaphase. (Holle and Harris 1992) Therefore the colchicine method is suitable for comparing the number of cells in metaphase of mitosis in different animals.

Schinkel (1961) observed a correlation between follicle bulb size and wool fibre size. Larger fibres were produced by larger bulbs and smaller fibres grew from smaller bulbs. Williams and Winston (1987) also found a positive correlation between bulb diameter and fibre cross-sectional area. Straile (1964) observed that the germinative area of the follicle bulb increased in size during the wool fibre growth cycle.

Hynd (1989) suggested that work needed to be carried out to relate changes in bulb size and mitotic activity within the same animal consuming different rations. In the present study the effect of changes in the nutritional state on bulb size and mitotic activity have been examined in sheep with a history of different staple strengths. Low and high staple strength sheep were subjected to a restricted ration followed by a low or high protein ration and data were collected to determine the effect of the nutritional state on the follicle bulb.

MATERIALS AND METHODS

2.1. Animals

Twelve Merino wethers (castrated rams) were selected from the sound and tender lines at the Great Southern Agricultural Research Institute (GSARI), Katanning. The sound lines are sheep that consistently produced high staple strength wool (>30 N/ktex) and the tender lines are sheep that consistently produced low staple strength wool (<25 N/ktex).

The sheep were from CSIRO protocol number 9204 which was approved by an animal care and ethics committee. Of the twelve sheep selected for this project, six were from the sound line (HSS, High Staple Strength) and six from the tender line (LSS, Low Staple Strength). The sheep were housed individually in holding pens at CSIRO Floreat for the duration of the experiment. The sheep were allowed water ad libitum throughout the experiment.

At the start of the experiment the sheep had a condition score of 3 on a scale from very lean (1) to very fat (5): the sheep were medium.

2.2. Diet

The diet manipulations of this experiment occurred over a period of eighteen weeks. During the first ten weeks all

sheep were fed on a restricted ration. During weeks eleven to eighteen the sheep were divided into two diet groups: high protein diet and low protein diet. Both diets were iso-energetic but with different protein composition. All sheep were weighed weekly.

2.2.1. Restricted Ration

Initially (0-10 weeks) sheep were fed a restricted ration to achieve a weight loss of 175 grams per day to reach a condition score of 1 (very lean) or less to simulate the late Autumn field situation in Western Australia.

Table 2.1. Components of the Restricted Ration

STRAW	48.3 %
HAY	47.8 %
MINERALS	2.2%
UREA-N	1.7%

Straw and hay provided roughage or bulk for the diet. The minerals (CSIROMIN) consisted of a full range of macro and micro elements to protect against any mineral deficiency. The urea provided fermentable nitrogen for the rumen to maintain efficacious levels of fermentation.

2.2.2. Low Protein and High Protein Rations

During weeks 11-18 sheep were fed at 1600 grams per day, one of two iso-energetic diets to achieve a weight gain of approximately 200 grams per day. The diets were equal in energy but different in protein content. One diet was a low protein ration 10% protein (diet 1) and the other a high protein ration 21% protein (diet 2).

Table 2.2. Components of the Low and High Protein Rations

COMPONENTS	HIGH PROTEIN (%)	LOW PROTEIN (%)
LUCERNE HAY	30.1	10.7
OATEN HAY	37.1	61.1
MINERALS	2.0	2.0
LUPINS	18.8	10.3
FISH MEAL	8.0	0.0
OATS GRAIN	0.0	11.5
UREA-N	0.6	0.0
STARCH	1.6	4.3
CaSO4	0.7	0.1

Lucerne and oaten hay provided good quality roughage. CSIRO MIN minerals ensured against mineral deficiency. Lupins provided high fermentable protein and energy whereas oats provided a safe source of starch for energy. Calcium sulphate ensures adequate calcium levels for normal functioning. Percentages of different components ensured that the diets were balanced for the sheep to achieve the required growth rates.

Twelve sheep were grouped according to staple strength (SS) and diet to give four groups:-

1. High SS, low protein diet 1 (n=3)
2. High SS, high protein diet 2 (n=3)
3. Low SS, low protein diet 1 (n=3)
4. Low SS, high protein diet 2 (n=3)

Animals were coded to ensure unbiased data collection.

2.3. Skin Samples

Skin samples were collected four hours after intradermal injections of colchicine 30 ug in 0.2 mL saline. Previous trials with colchicine had established that 30 ug was a suitable non-toxic dose and four hours was the optimum time on a time response curve to obtain accurate estimation of the number of cells entering metaphase (personal communication Schlunk). Colchicine is a strathmokinetic agent which arrests cells in the metaphase stage of cell division (Hynd et al 1986). Mid-side skin biopsies were

collected with a 1 cm trephine following injection of 0.5 ml local anaesthetic (Lignocaine). Samples were fixed in Serra's fixative (Maddocks and Jackson 1988) for five hours then stored in seventy per cent ethanol until processed.

The experiment ran for eighteen weeks and samples were collected from each sheep on each of six dates:

Diet	Week	Sample
Pre-treatment	0	Date 1
Restricted Ration	2	Date 2
	10	Date 3
High or Low Protein	11	Date 4
	12	Date 5
	18	Date 6

2.4. Histology

Samples were embedded in paraffin with a vertical orientation, so that the epidermal (surface) layer of the skin was perpendicular to the face of the paraffin block. 8 um serial, vertical sections were cut using a rotary microtome. A minimum of fifty serial sections were collected for each block to ensure sufficient numbers of follicles would be available for counting. The sections were picked up on glass slides to which a drop of glycerine

albumen slide adhesive (Maddocks and Jackson 1988) had been applied. The total number of blocks sectioned was 72 (i.e. 6 sample times per 12 animals), with subsequent staining of at least 3600 tissue sections.

2.5. Staining

Sections were stained using a modified saccpic stain as follows. (Personal communication Hynd 1992)

Xylene	2 x 10 mins
100% Ethanol	2 x 2 mins
90% Ethanol	2 mins
70% Ethanol	2 mins
Distilled water	2 mins
Lillee Mayer Haematoxylin	10 mins
Tap water	30 secs
70% Ethanol	30 secs
Winiwater's Safranin	15 mins
70% Ethanol	6 secs
100% Ethanol	30 secs
Picric Acid	5 secs
Tap water	30 secs
Distilled water	30 secs
Picro-Indigo Carmine	2 mins
70% Ethanol	7 secs
100% Ethanol	2 x 2 mins
Xylene	2 x 2 mins
Mounted in Depex	

2.6. Quantitative Observations

Paraffin blocks were numbered by code and not date or type of treatment to ensure the elimination of observational bias.

2.6.1. Mitotic Counts

To estimate the mitotic rate in the follicular bulb the method of Schinkel (1961) was used. The number of metaphase arrested cells per follicle per animal was counted in 100 follicles per animal. Only every fourth section (of serial sections) was counted to ensure against counting the same follicle twice. The metaphase arrested cells appeared as dark blue/black stained cells. The counts were carried out at 100x magnification using an Olympus CO11 microscope.

2.6.2. Criteria for Selecting Follicular Bulbs

1. A visible dermal papilla, seen as a green stained area within the follicle bulb.
2. Oval shaped follicle bulb to ensure transverse plane.
3. Zone of differentiation, seen as an area with elongated cells, superior to dermal papilla.

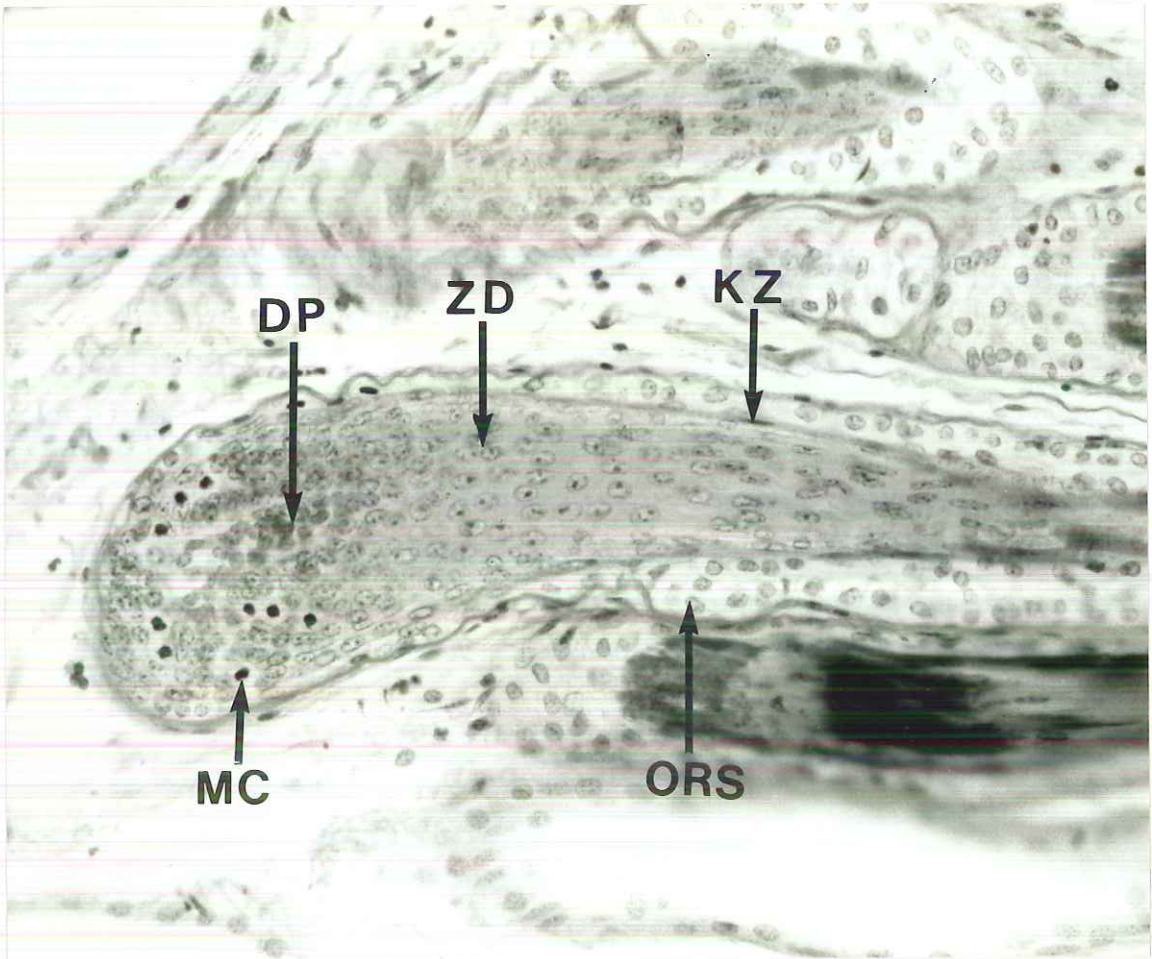
Figure 2.1 shows a representative follicle with the following structures labelled: mitotic cell; dermal papilla; zone of differentiation; keratogenous zone; outer root sheath bulge.

For each follicle which met the criteria the number of mitotic cells per follicle was recorded for one hundred follicles per animal per date of treatment. For each sampling time a minimum of 100 follicle bulbs that met the criteria were counted to determine the number of metaphase arrested cells per follicle bulb.

2.7. Area of Follicle Bulb

Hocking Edwards and Hynd (1992) defined the area of the germinative region of the wool follicle bulb as the total area of the follicle bulb terminating in a line one cell width above the dermal papilla. The area was measured in follicle bulbs which met the criteria for inclusion in the mitotic counts. The area in which the metaphase arrested cells were found was measured in μm^2 . Twenty five follicles per sheep per group per treatment were counted which represents a total of six hundred follicles measured. Measurements were carried out using a Chromatic Colour Image Analysis System, Version 2.2 Jarvis L.R. (1990) and a Leitz microscope at 200x magnification.

Figure 2.1. A Representative Follicle for Counting



MC = Mitotic Cell

DP = Dermal Papilla

ZD = Zone of Differentiation

KZ = Keratogenous Zone

ORS = Outer Root Sheath Bulge

Two animals were selected at random from each of the four experimental groups and twenty five follicle bulbs per animal were measured. Coded samples from the following dates were selected:

Date 1. Pre-treatment.

Date 3. Ten weeks on restricted ration.

Date 6. Eight weeks on high or low protein diet.

The following gross parameters (clean wool weight and fibre diameter) were obtained by CSIRO and are used to relate to the morphological data obtained in this Honours project.

2.8. Clean Wool Weight

Marked patches of wool were collected, with clippers and weighed. The greasy wool was then washed, air dried and weighed. The clean wool weight was calculated by multiplying the greasy wool weight by the yield to give grams of wool produced per patch. The patch size varied from 10,282 mm² to 13,868 mm², mean 12,192 mm².

2.9. Fibre Diameter

To measure the fibre diameter cleaned snippets of wool were dried and spread on a glass microscope slide. The slides were placed onto the microscope stage of an optical fibre diameter analyser which measured and recorded the fibre diameters in microns as well as coefficients of variation.

The coefficient of variation measures the spread of fibre diameter variation relative to the average. The lower the coefficient of variation, the narrower and more uniform the fibre diameter distribution.

2.10. Statistical Methods

The Kolmogorov-Smirnov (K-S) test was used to compare samples with a non-normal distribution. It is a two tailed test which can be used to determine whether two samples have been drawn from the same population or from two populations of the same distribution. A large difference in cumulative distribution indicates that the two samples are significantly different. To apply the K-S two sample test a cumulative frequency distribution is made for the two samples. The test focuses on the largest difference between the two cumulative frequencies (Siegel 1956).

Analysis of variance according to staple strength and diet and multiple regression were carried out using Minitab.

t-tests on the means of mitotic counts, follicle bulb areas and weights were carried out using Mynstat.

2.11. Qualitative Observations

Qualitative observations were made to determine the stages of the growth cycle of the wool fibre. The stage of the growth cycle was determined based on the presence or

absence of the features described below. Descriptions published by Straile et al (1961), Parakkal (1970, 1990) and Maddocks and Jackson (1988) were used to list criteria for identification of each stage of the growth cycle.

Two animals were selected from each of the four experimental groups and twenty five bulbs per animal were measured. Only those follicles in the anagen or telogen stage of the growth cycle could be identified with any confidence. Follicles which did not fit the criteria listed for a particular stage were not classified.

Anagen, the active or proliferative stage of the growth cycle, (Figure 2.1) was indicated by the following features:

1. Enclosure of the dermal papilla in the follicle bulb.
2. Keratin (bright red stain) present in the lower shaft or superior portion of the follicle.
3. Outer root sheath bulge indicating the rapid division of cells in the follicle bulb.
4. Presence of blood vessels in the dermal papilla.

Catagen, the transition or retrogressive stage of the growth cycle, (Figure 2.2) was indicated by:

1. Shortening of the follicle.
2. Decrease and final cessation of mitosis.

3. Breakdown of the blood vessels in the dermal papilla.

Telogen, the resting phase, (Figure 2.3) was indicated by:

1. Follicles 1/3 of anagen length i.e. closer to the epidermal layer.
2. No matrix, inner root sheath or outer root sheath.
3. Dermal papilla present only as a ball of cells.
4. Club end from previous cycle may be present.

Each follicle was examined for the following features to determine the stage of the cell cycle:

1. Whether an outer root sheath bulge was present.
2. Whether vessels were present in the dermal papilla.
3. Whether keratin was present in the lower shaft or superior follicle region.

Figure 2.2. Diagram of an anagen follicle. (After Parakkal 1990)

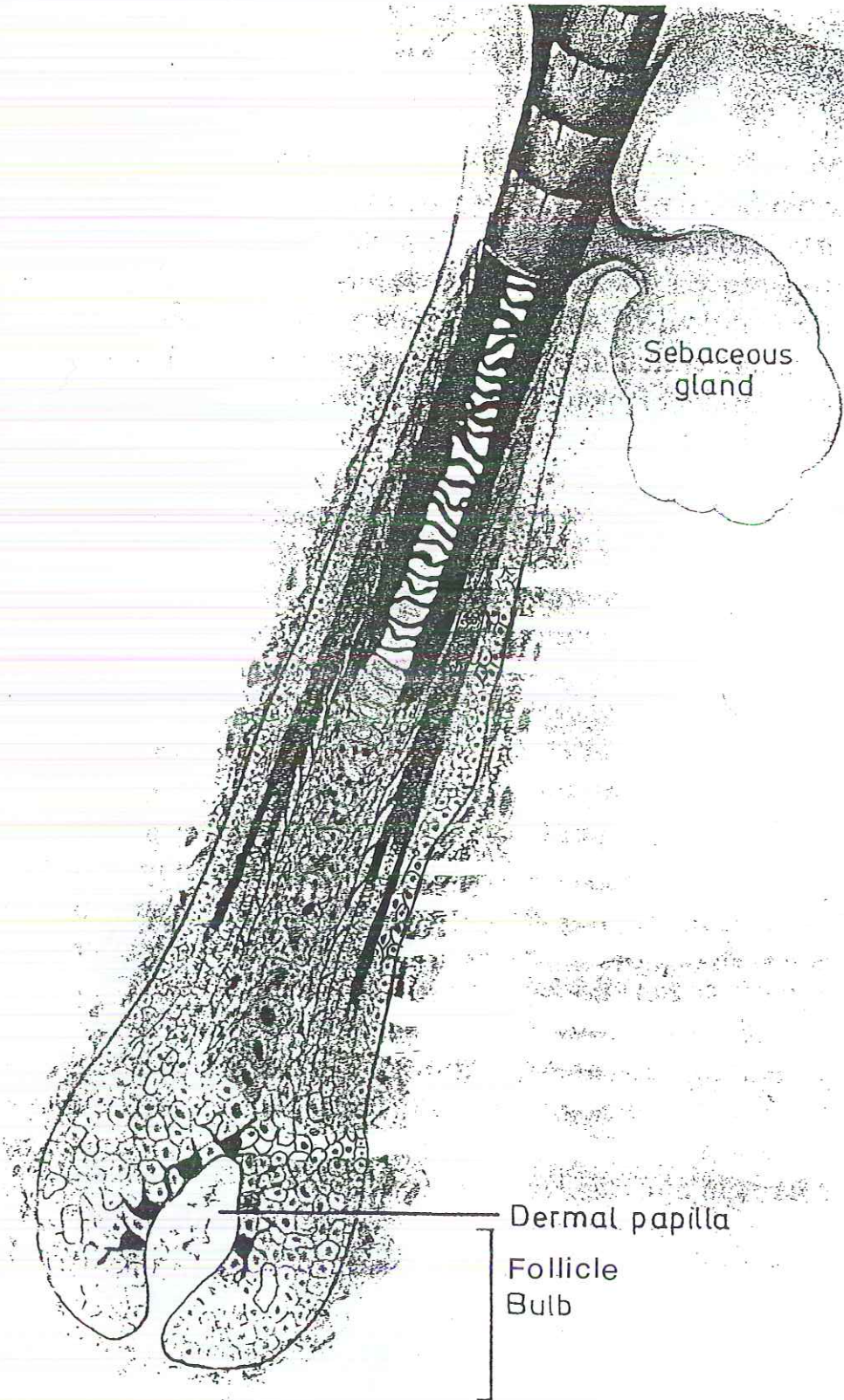


Figure 2.3. Diagram of a catagen follicle. (After Parakkal 1990)

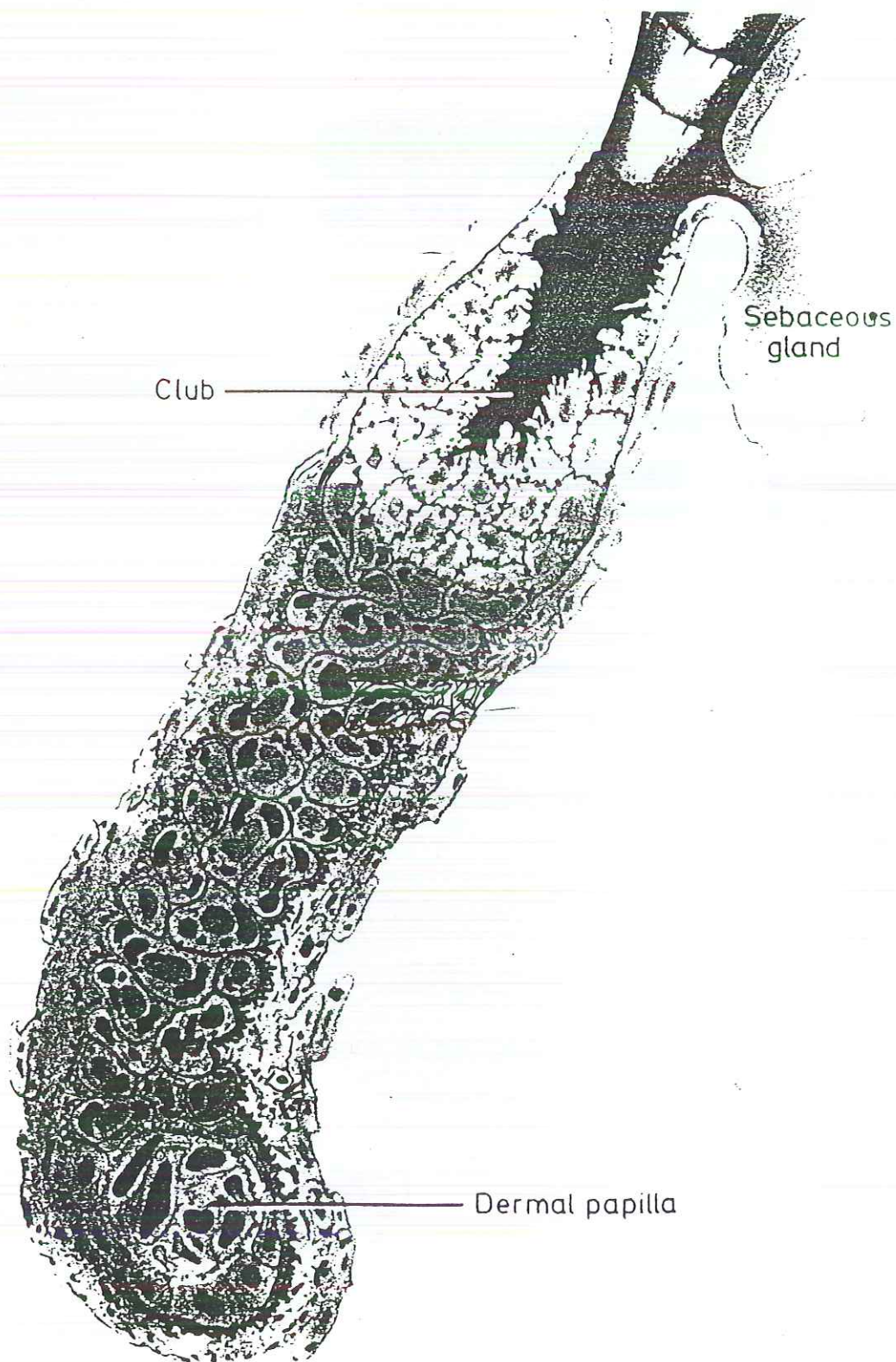
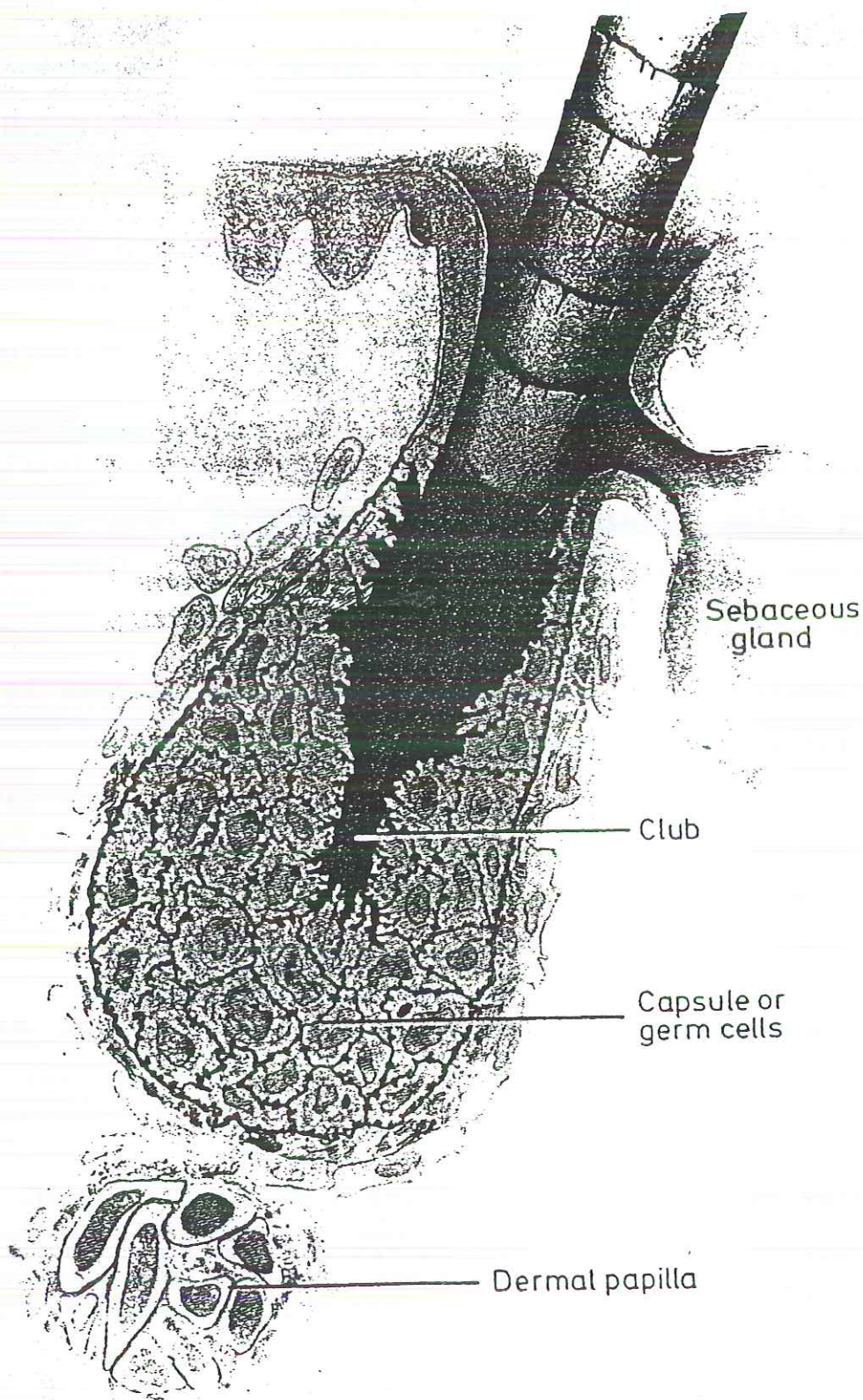


Figure 2.4. Diagram of a telogen follicle. (After Parakkal
1990)



RESULTS

3.1. Quantitative

3.1.1. Frequency Distribution of Mitotic Cells in Follicle Bulbs (Figures 3.1-3.6)

Figures 3.1-3.6 show the frequency distributions for mitotic count per follicle per group on each of six sampling dates.

In the first ten weeks of the experiment (Figures 3.1-3.3), a shift in peak frequency from 4-6 mitotic cells per follicle bulb to 2-3 mitotic cells per follicle bulb can be seen indicating a slowing of cell division. After the change of diet to the low or high protein ration (Figures 3.4-3.6), the peak frequency has shifted from 5-7 mitotic cells per follicle bulb which is slightly higher than the pre-treatment frequency of 4-6 mitotic cells per follicle bulb.

There is also a tailing of the frequency distribution towards the right, with more mitotically active cells per follicle bulb after eight weeks on the low or high protein ration. This shift to the right is evident after two weeks on the low or high protein ration and there is no apparent difference between the low and high protein groups (Figure 3.5).

Figure 3.1. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 0 PRE-TREATMENT

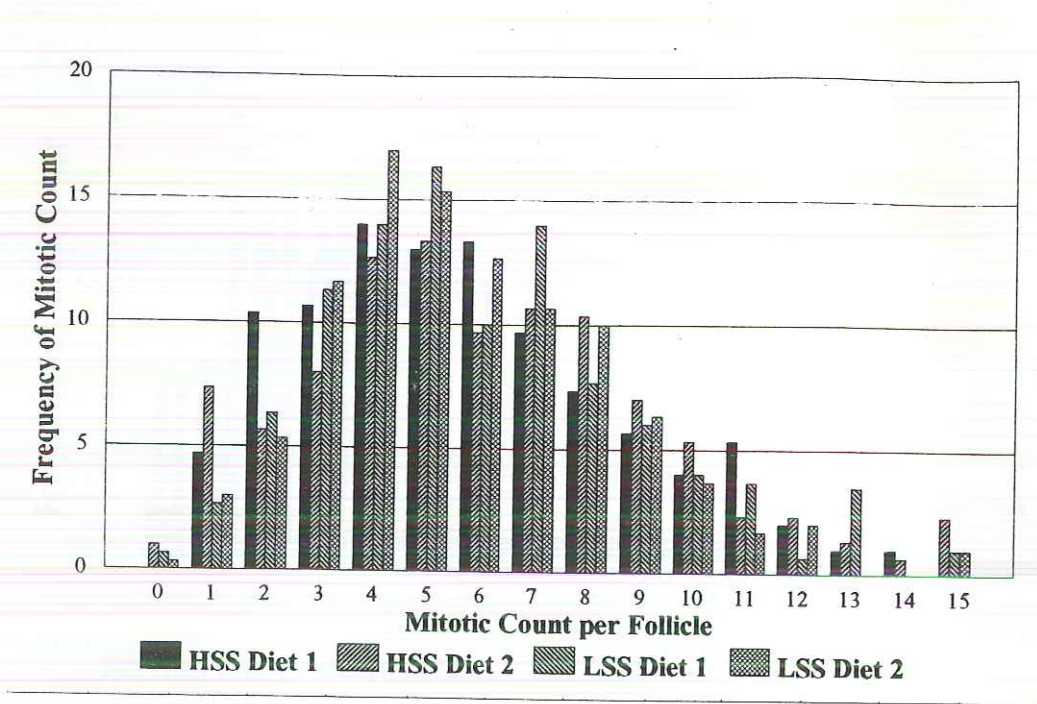


Figure 3.2. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 2 TWO WEEKS ON RESTRICTED RATION

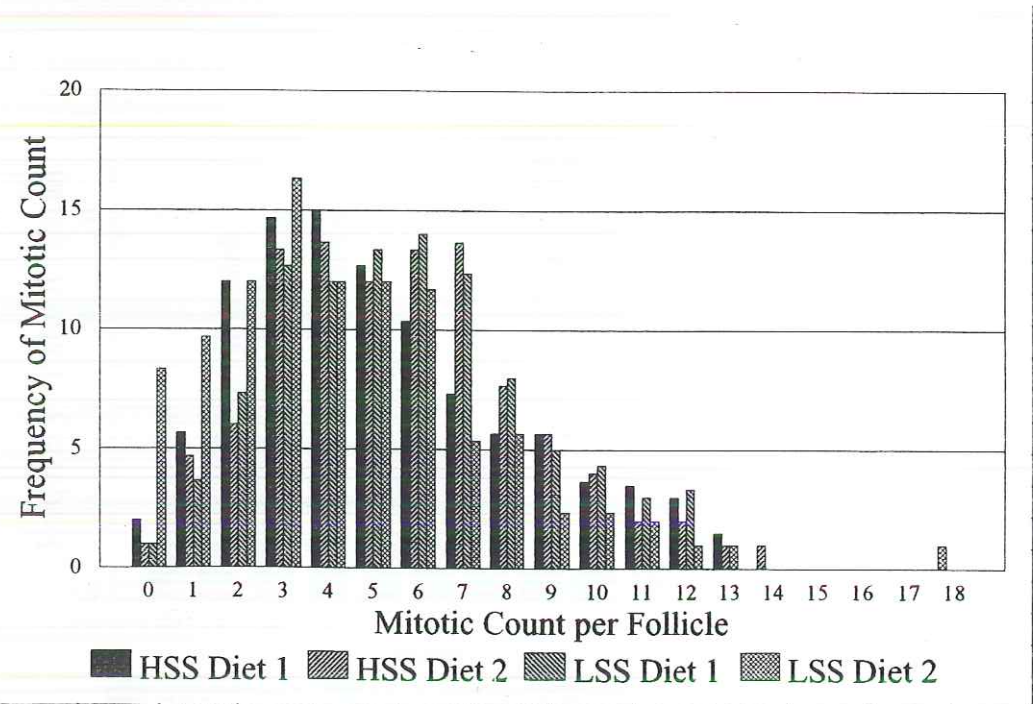


Figure 3.3. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 10 TEN WEEKS ON RESTRICTED RATION

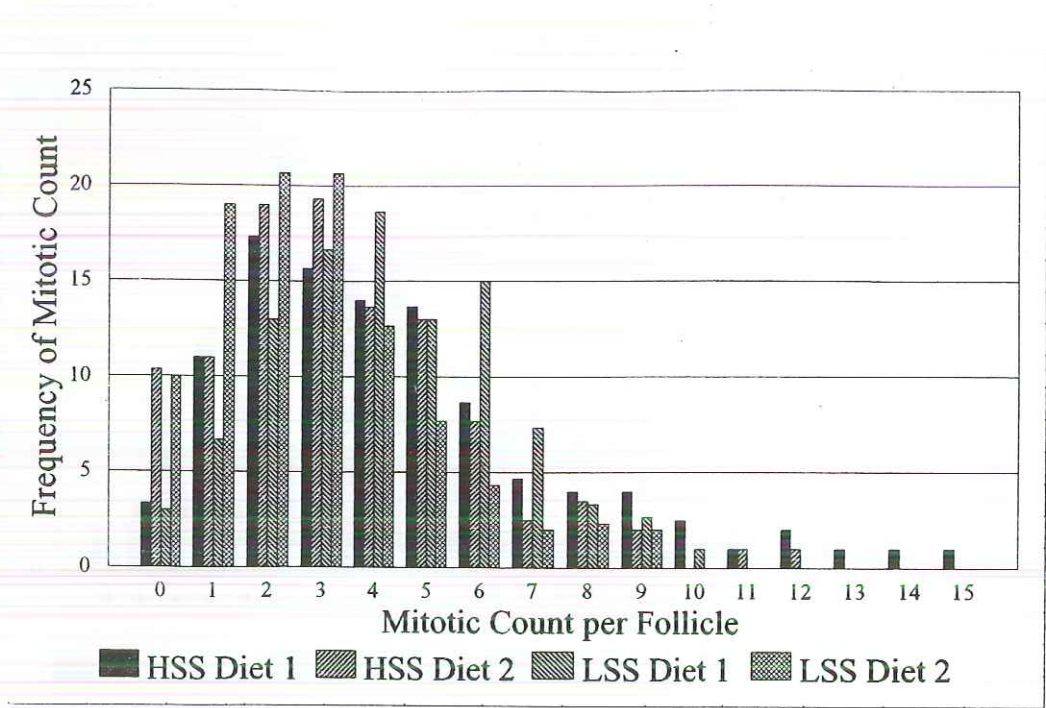


Figure 3.4. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 11 ONE WEEK ON LOW OR HIGH PROTEIN RATION

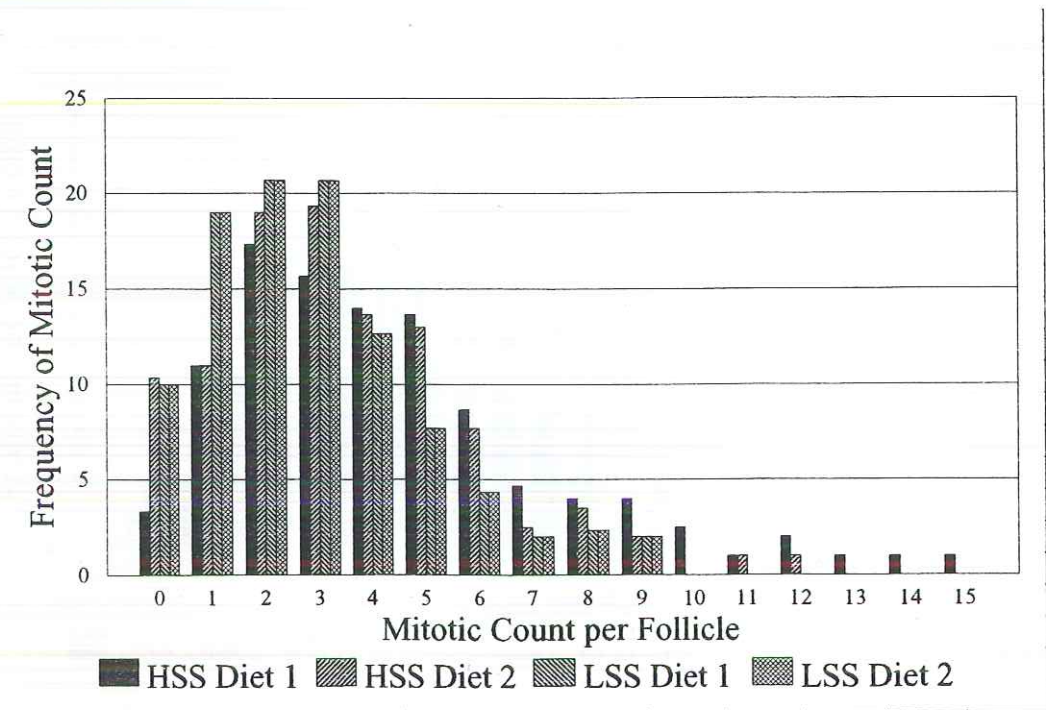


Figure 3.5. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 12 TWO WEEKS ON LOW OR HIGH PROTEIN RATION

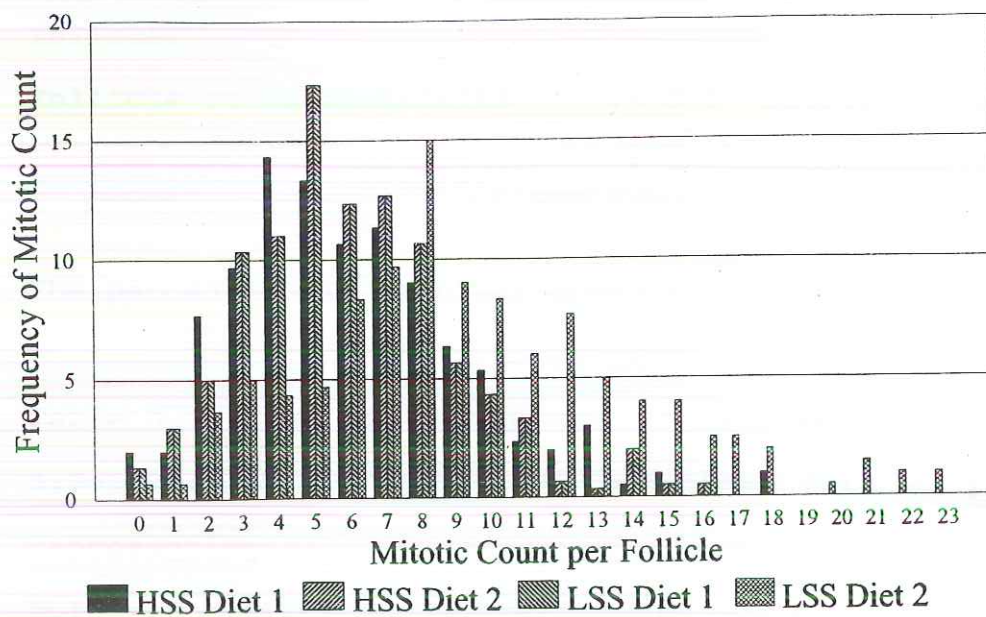
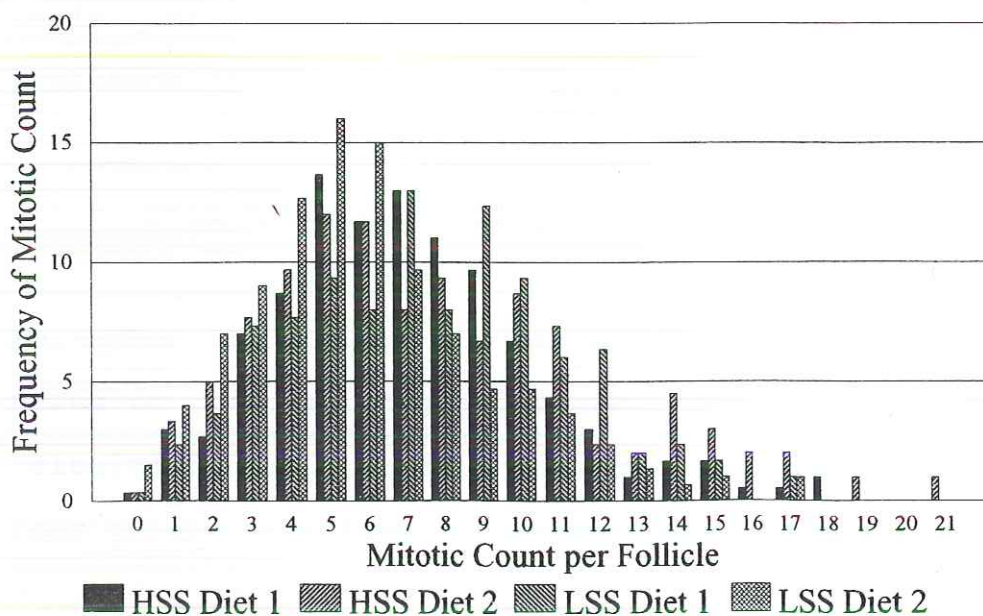


Figure 3.6. Frequency Distribution of Mitotic Cells in Follicle Bulbs

WEEK 18 EIGHT WEEKS ON LOW OR HIGH PROTEIN RATION



3.1.2. Range of Mitotic Counts (Table 3.1, Figures 3.7-3.9)

In order to clarify the frequency distribution, the mitotically active cells per follicle were sub-grouped as follows:

- | | |
|---------------------------|--------------------------|
| Follicle bulbs containing | - 0-2 mitotic cells, |
| | - 3-11 mitotic cells and |
| | - 12+ mitotic cells. |

The percentage in each sub-group was then estimated.

Table 3.1 shows the sub-groups in tabular form and Figures 3.7-3.9 show the results in graphical form as pie charts representing the percentage of mitotic cells per follicle bulb per group per treatment. Figure 3.8 clearly shows the high incidence of follicle bulbs with 0-2 mitotic cells per follicle bulb. This represents a slowing of cell division at the end of ten weeks on the reduced ration. Figure 3.9 shows that the frequency of follicle bulbs with 12+ mitotic cells per follicle bulb has increased from the pre-treatment values (Figure 3.7).

3.1.3. Mean Mitotic Counts (Table 3.2, 3.3, Figure 3.10)

In order to examine the analysis of variance over time based on diet and staple strength mean mitotic counts were calculated (Table 3.2). The mean mitotic counts for the four groups over time are represented graphically in Figure 3.10. Repeated analysis of variance of the means (Table

3.3) of the four groups for each date showed no significant difference. ($p = 0.1-1.0$) However, t-tests performed on each sampling date showed significant differences between week 0 (pre-treatment) and week 10 (10 weeks on reduced ration) $T=5.75$ $p=0.000$; between week 0 and week 18 (eight weeks on low or high protein diet) $T=-2.39$ $p=0.036$; between week 10 and week 18 $T=-6.04$ $p=0.000$.

The results of the Kolmogorov-Smirnov test on mitotic counts indicated that the animals in the High Staple Strength Diet 2 group and the Low Staple Strength Diet 2 group were similar statistically ($>10\%$ chance of coming from the same population). However the animals in the High Staple Strength Diet 1 and Low Staple Strength Diet 1 groups were not statistically similar ($<0.5\%$ chance of coming from the same population).

Table 3.1. Percentage of Mitotic Counts in a Range

WEEK 0 PRE-TREATMENT

	0-2	3-11	12+
HSS DIET 1	10	87	3
HSS DIET 2	9	89	2
LSS DIET 1	15	83	2
LSS DIET 2	14	79	7

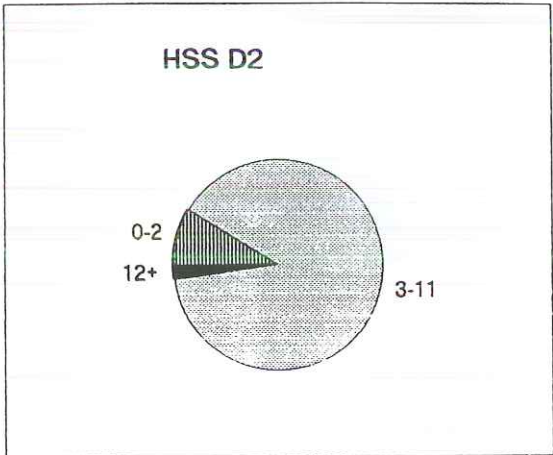
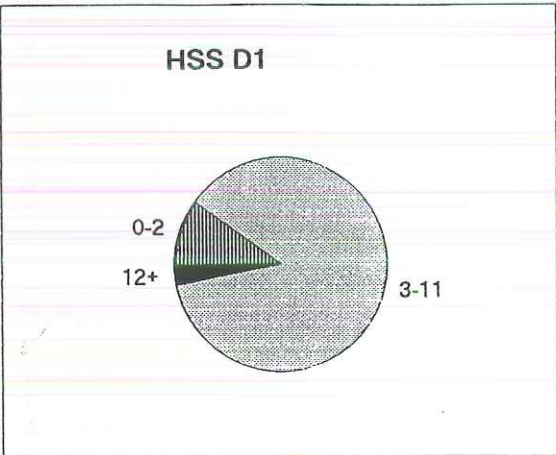
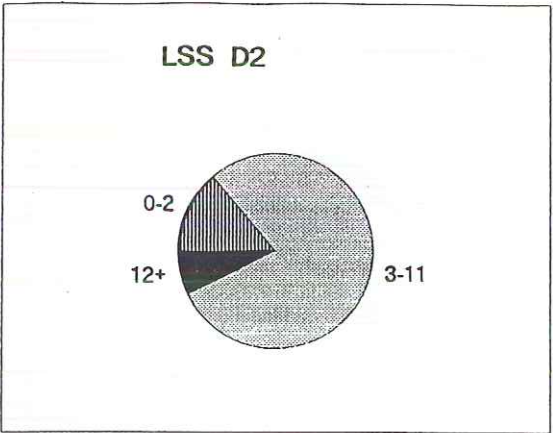
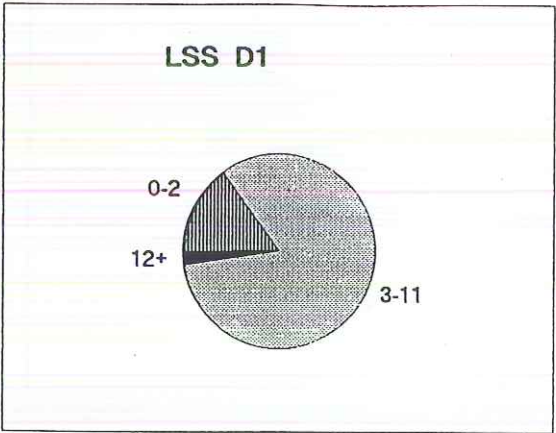
WEEK 10 EIGHT WEEKS ON RESTRICTED RATION

	0-2	3-11	12+
HSS DIET 1	23	77	0
HSS DIET 2	50	50	0
LSS DIET 1	32	67	1
LSS DIET 2	40	59	1

WEEK 18 EIGHT WEEKS ON LOW OR HIGH PROTEIN RATION

	0-2	3-11	12+
HSS DIET 1	6	81	13
HSS DIET 2	12	82	6
LSS DIET 1	6	86	8
LSS DIET 2	9	81	10

Figure 3.7. Percentage of Mitotic Counts per Follicle bulb
in a Range. Week 0 Pre-Treatment



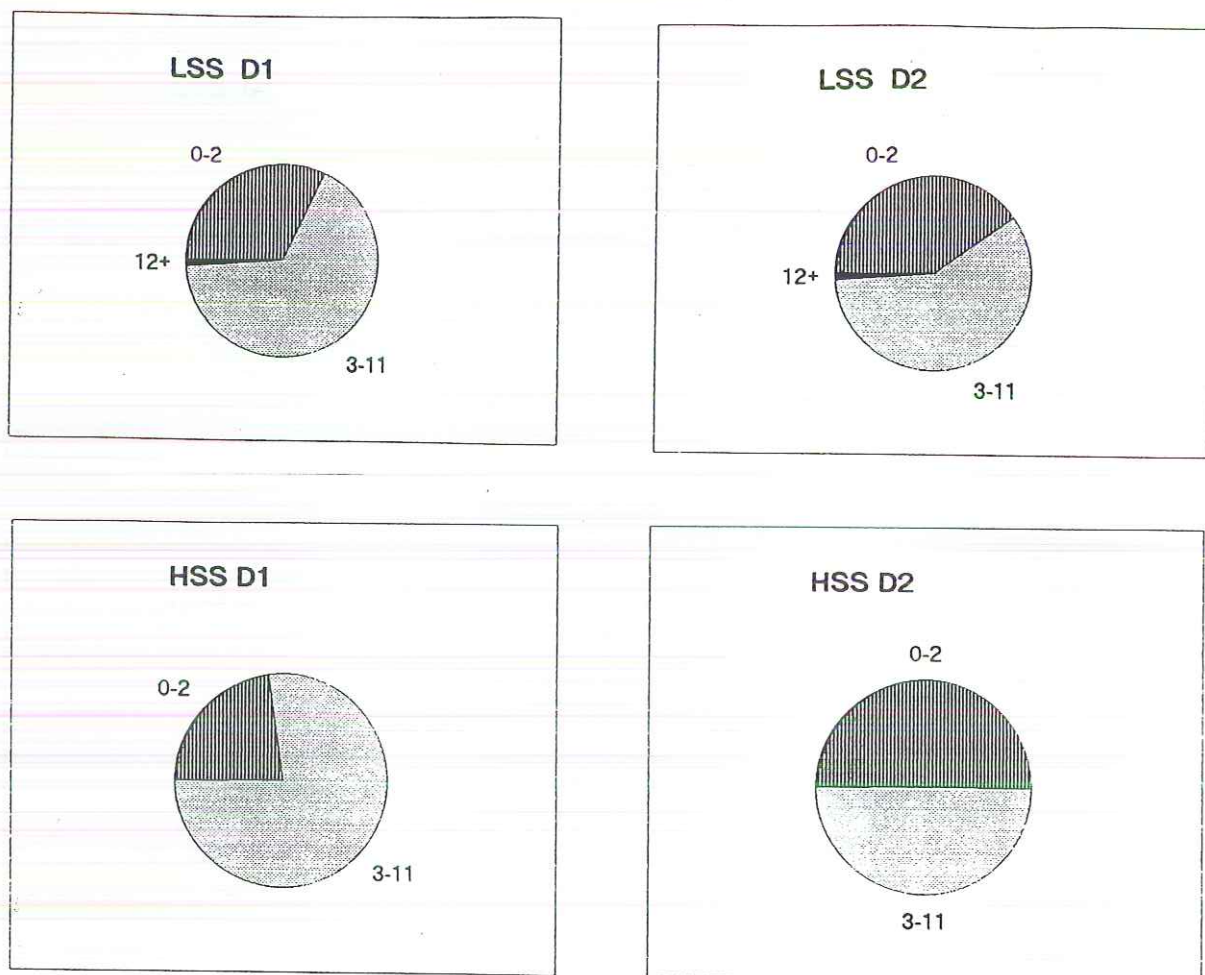
HSS D1 = High staple strength low protein.

HSS D2 = High staple strength high protein.

LSS D1 = Low staple strength low protein.

LSS D2 = Low staple strength high protein.

Figure 3.8. Percentage of Mitotic Counts per Follicle bulb
in a Range. Week 10 Ten Weeks on Restricted
Ration



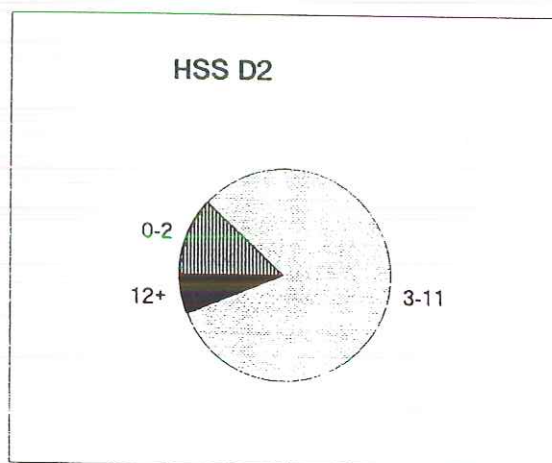
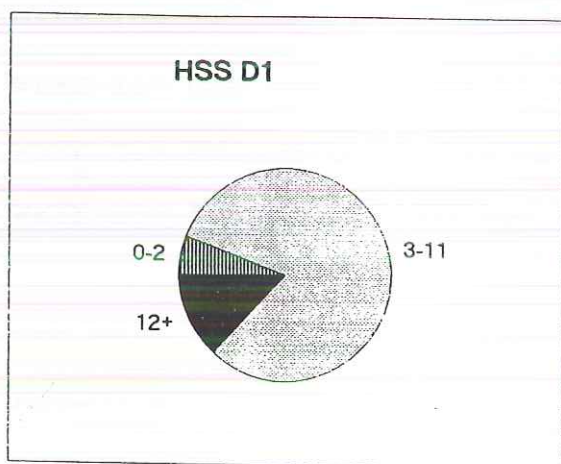
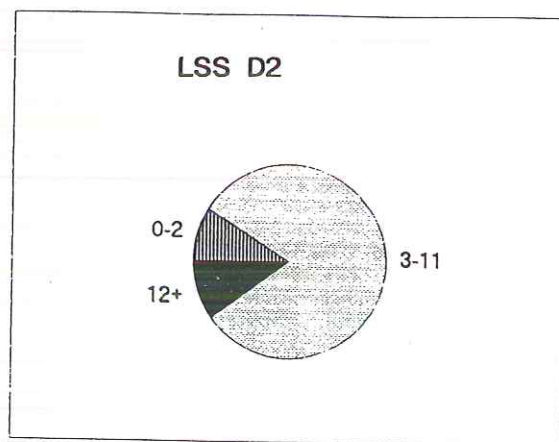
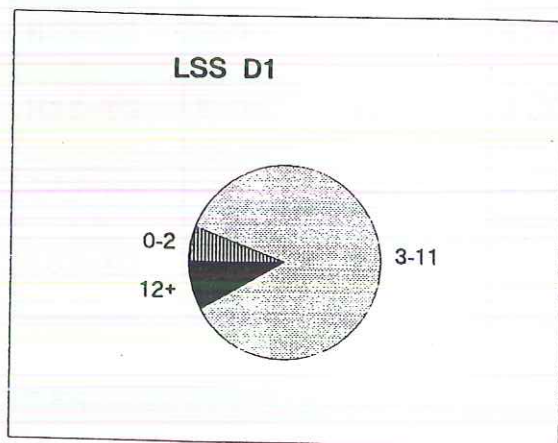
HSS D1 = High staple strength low protein.

HSS D2 = High staple strength high protein.

LSS D1 = Low staple strength low protein.

LSS D2 = Low staple strength high protein.

Figure 3.9. Percentage of Mitotic Counts per Follicle bulb
in a Range. Week 18 Eight Weeks on Low or High
Protein Ration



HSS D1 = High staple strength low protein.

HSS D2 = High staple strength high protein.

LSS D1 = Low staple strength low protein.

LSS D2 = Low staple strength high protein.

Table 3.2. Pooled Mean Mitotic Count per Group per Date

	WEEK 0	WEEK 2	WEEK 10	WEEK 11	WEEK 12	WEEK 18
HSS-D1	5.7	5.7	4.3	6.0	6.0	7.3
HSS-D2	5.7	4.3	2.3	6.0	8.7	6.0
LSS-D1	5.7	5.3	4.3	5.3	6.3	7.0
LSS-D2	5.7	5.3	3.7	5.7	7.7	7.3

WEEK 0 = Pre-treatment.

WEEK 2 = Two weeks on restricted ration.

WEEK 10= Ten weeks on restricted ration.

WEEK 11= One week on low high protein ration.

WEEK 12= Two weeks on low or high protein ration.

WEEK 18= Eight weeks on low or high protein ration.

HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Table 3.3. Analysis of Variance for Mean Mitotic Counts

SOURCE SS x DIET	DF	SS	MS	F	P
WEEK 0	3	0.0	0.0	0.0	1.0
WEEK 2	3	3.6	1.2	2.1	0.2
WEEK 10	3	8.0	2.7	3.2	0.1
WEEK 11	3	0.9	0.3	0.3	0.9
WEEK 12	3	16.9	5.6	2.6	0.1
WEEK 18	3	3.6	1.2	0.3	0.8

WEEK 0 = Pre-treatment.

WEEK 2 = Two weeks on restricted ration.

WEEK 10= Ten weeks on restricted ration.

WEEK 11= One week on low high protein ration.

WEEK 12= Two weeks on low or high protein ration.

WEEK 18= Eight weeks on low or high protein ration.

DF = Degrees of freedom.

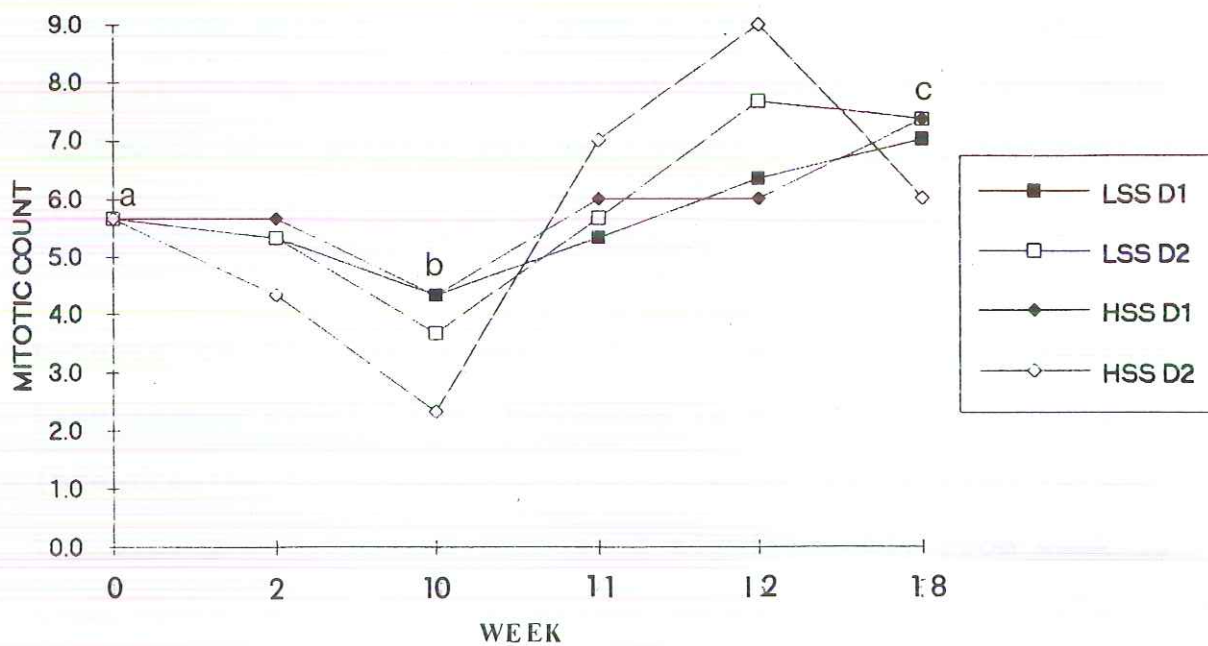
SS = Sum of squares.

MS = Mean squares.

F = The variance ratio.

P = Probability.

Figure 3.10. Follicular Bulb Mean Mitotic Cell Count per
Group per Date



Subscripts a,b and c denote group means that are statistically different at $p < 0.05$. That is ab, ac and bc.

3.1.4. Follicle Bulb Area (Table 3.4, Figure 3.11)

The follicle bulb (germinative) area was measured in two sheep from each group for week 0 (pre-treatment), week 10 (ten weeks on restricted ration) and week 18 (eight weeks on low or high protein ration). Table 3.4 shows the pooled mean germinative area in um^2 .

t-tests on the group means for germinative area against time showed significant decreases in all groups from week 0 (pre-treatment) to week 10 (ten weeks on restricted ration) $t = 5.87$ $p = 0.010$ and increased significantly from week 10 (ten weeks on restricted ration) to week 18 (eight weeks on low or high protein ration) $t = -15.704$ $p = 0.001$. Table 3.4 shows the mean areas per group per date. Figure 3.11 shows a graphical representation of the data.

Table 3.4. Pooled Mean Follicle Bulb Area (μm^2) per Group
per Date

	WEEK 0 MEAN (SEM)	WEEK 10 MEAN (SEM)	WEEK 18 MEAN (SEM)
HSS-D1	9,945 (446)	6,712 (308)	10,728 (489)
HSS-D2	8,108 (293)	6,424 (191)	10,330 (315)
LSS-D1	8,068 (298)	6,277 (226)	9,834 (303)
LSS-D2	9,183 (347)	7,307 (290)	10,299 (313)

WEEK 0 = Pre-treatment.

WEEK 10 = Ten weeks on restricted ration.

WEEK 18 = Eight weeks on low or high protein.

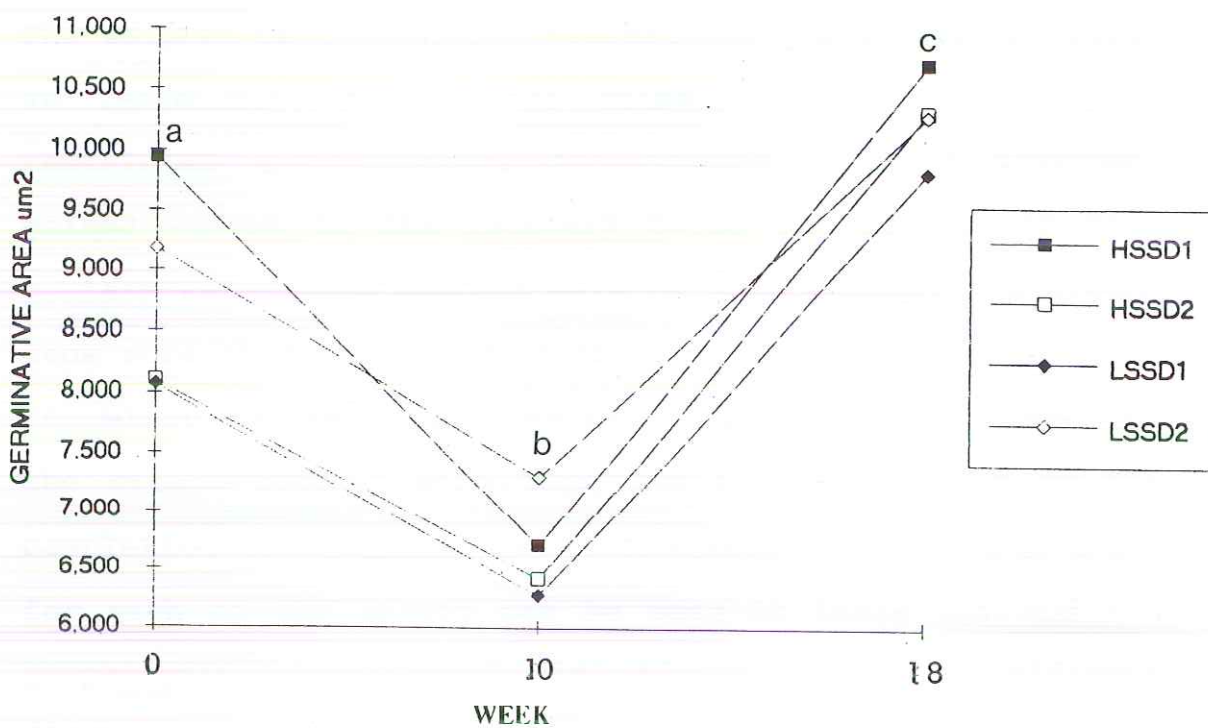
HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Figure 3.11. Follicle Bulb Area per Group per Date



Subscripts a,b and c denote group means that are statistically different at $p < 0.05$. That is ab and bc.

3.1.5. Live-weight (Table 3.5, 3.6, Figure 3.12)

The weights of the animals for each of the groups are shown in Table 3.5. All of the animals lost weight with a restricted energy intake (an average loss of 31%) and gained weight in the recovery phase on the low or high protein ration (an average gain of 33%). All of the groups took more than two weeks on the low or high protein ration to return to their pre-treatment weight. Sheep in each of the groups weighed more than the starting weight at the completion of the experiment. The weight losses and gains for each of the groups can be seen in Table 3.6 and are graphically represented in Figure 3.12. Significant differences were found between the weights per group for the following weeks: 0 and 2, 0 and 10, 10 and 11, 10 and 18 ($p < 0.05$).

3.1.6. Clean Wool Weight (Table 3.7, Figure 3.13)

The clean wool weights can be seen in Table 3.7 and in graphical form in Figure 3.13. The amount of wool produced fell after ten weeks on the restricted ration. Initially 0.55 - 0.66 g/10,000 mm² were produced but after ten weeks on the restricted ration production had fallen to 0.20 - 0.24 g/10,000 mm². After eight weeks on the low or high protein ration the amount of wool produced had risen to 0.96 - 1.41 g/10,000 mm² which was higher than pre-treatment.

Table 3.5. Pooled Weight (kg) per Group per Date

	WEEK 0 (SEM)	WEEK 2 (SEM)	WEEK 10 (SEM)	WEEK 11 (SEM)	WEEK 12 (SEM)	WEEK 18 (SEM)
HSS- D1	59 (2.7)	57 (2.7)	54 (2.7)	57 (2.6)	58 (2.7)	62 (2.7)
HSS- D2	57 (2.2)	55 (2.1)	52 (1.7)	55 (1.8)	56 (1.7)	62 (2.3)
LSS- D1	60 (2.6)	56 (3.5)	53 (3.2)	57 (3.4)	58 (3.2)	63 (2.4)
LSS- D2	60 (3.1)	58 (3.0)	55 (2.4)	57 (2.4)	58 (2.3)	64 (3.0)

WEEK 0 = Pre-treatment.

WEEK 2 = Two weeks on restricted ration.

WEEK 10= Ten weeks on restricted ration.

WEEK 11= One week on low high protein ration.

WEEK 12= Two weeks on low or high protein ration.

WEEK 18= Eight weeks on low or high protein ration.

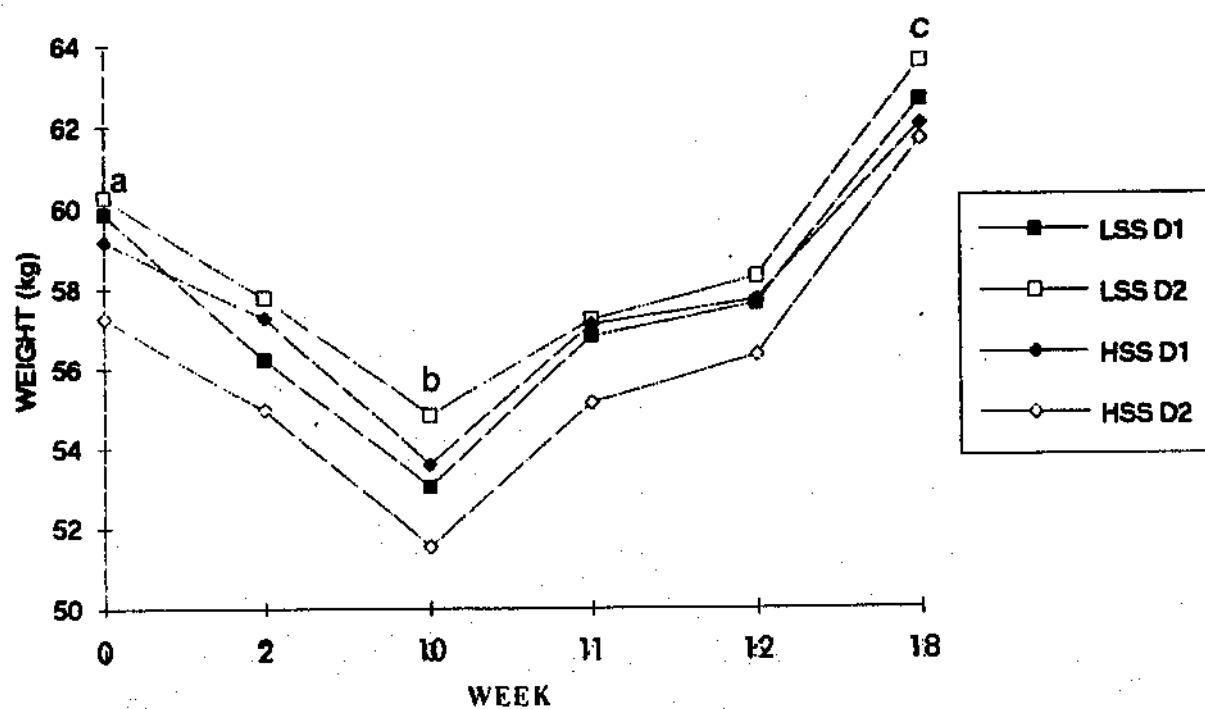
HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Figure 3.12. Live-weight (kg) per Group per Date



Subscripts a,b and c denote group means that are statistically different at $p < 0.05$. That is ab, ac and bc.

Table 3.6. Net Gains and Losses in Weight (kg) per Group
per Date

	WEEK 2	WEEK 10	WEEK 11	WEEK 12	WEEK 18
HSS-D1	-1.9	-5.6	-2.1	-1.5	2.8
HSS-D2	-2.3	-5.7	-2.1	-2.8	4.4
LSS-D1	-3.6	-6.8	-3.1	-2.3	2.7
LSS-D2	-2.5	-5.4	-3.1	-2.0	3.3
MEAN	-2.6	-5.9	-2.6	-2.1	3.3
(SEM)	(0.2)	(0.2)	(0.2)	(0.2)	(0.2)

WEEK 2 = Two weeks on restricted ration.

WEEK 10= Ten weeks on restricted ration.

WEEK 11= One week on low high protein ration.

WEEK 12= Two weeks on low or high protein ration.

WEEK 18= Eight weeks on low or high protein ration.

HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Table 3.7. Clean Wool Weight (g/10,000 mm²) per Group per Date

	WEEK 0 (SEM)	WEEK 10 (SEM)	WEEK 18 (SEM)
HSS-D1	0.55 (0.06)	0.20 (0.04)	0.96 (0.28)
HSS-D2	0.57 (0.06)	0.21 (0.02)	1.41 (0.23)
LSS-D1	0.66 (0.10)	0.24 (0.11)	1.04 (0.21)
LSS-D2	0.59 (0.06)	0.21 (0.03)	1.23 (0.06)

WEEK 0 = Pre-treatment.

WEEK 10 = Ten weeks on restricted ration.

WEEK 18 = Eight weeks on low or high protein ration.

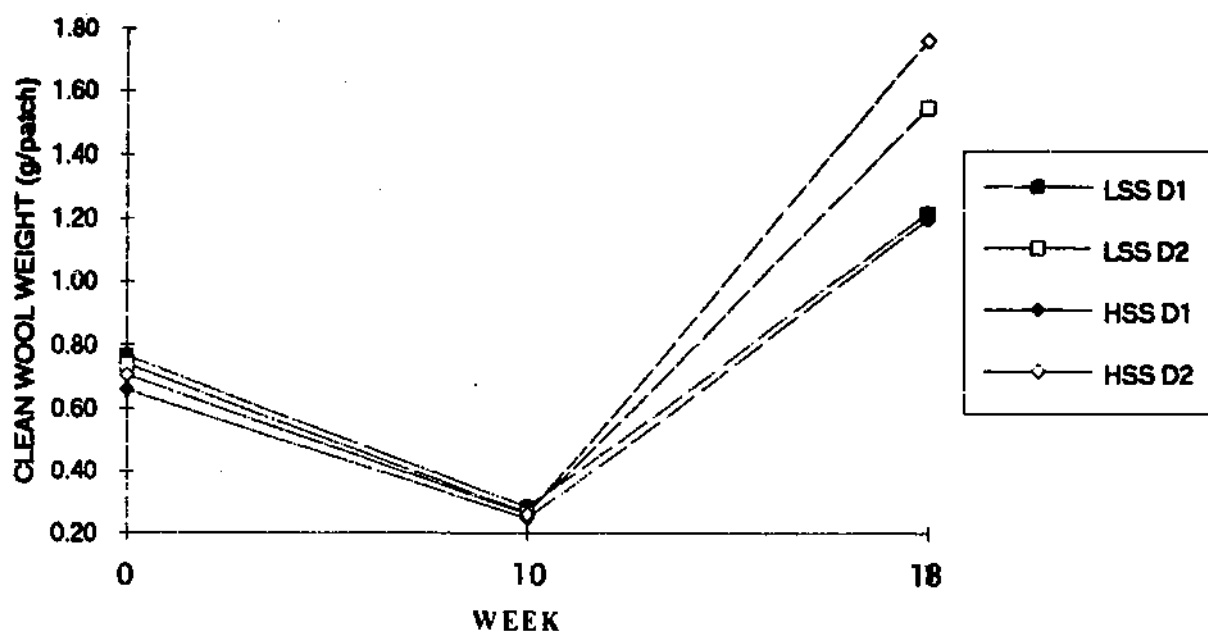
HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Figure 3.13 Clean Wool Weight (g/10,000 mm²) per Group per
Date



3.1.7. Fibre Diameter (Table 3.8, Figure 3.14)

The fibre diameters and coefficients of variation can be seen in Table 3.8. Figure 3.14 shows the graphical representation of the fibre diameter from week 0 to week 18. Pre-treatment the fibre diameters ranged from 17.2 - 18.8 μm . After ten weeks on the restricted ration they had narrowed to 14.7 - 15.9 μm , a loss of approximately 4 μm . After eight weeks on the low or high protein ration the fibre diameters had widened to 20.5 - 24.1 μm , a gain of approximately 8 μm from the end of the period on restricted ration and approximately 4 μm more than pre-treatment. In each of the groups except HSS diet 1 the CV was increased at the end of the restricted ration by an average of 2 %. The HSS diet 1 group CV remained unchanged during the restricted ration period. By the end of eight weeks on the low or high protein ration the CV for each group had returned to an average of 2 % less than the pre-treatment CV.

**Table 3.8. Fibre Diameter (um) and Coefficient of Variation
per Group per Date**

	WEEK 0 (SEM)	WEEK 10 (SEM)	WEEK 18 (SEM)	CV 0	CV 10	CV 18
HSS-D1	18.4 (0.35)	14.9 (0.41)	22.7 (0.62)	22	22	19
HSS-D2	18.7 (0.24)	14.8 (0.50)	24.1 (0.49)	16	19	18
LSS-D1	17.2 (0.67)	14.7 (0.72)	20.5 (0.64)	21	22	19
LSS-D2	18.8 (0.36)	15.9 (0.38)	22.7 (0.32)	22	23	21

WEEK 0 = Pre-treatment.

WEEK 10 = Ten weeks on restricted ration.

WEEK 18 = Eight weeks on low or high protein ration.

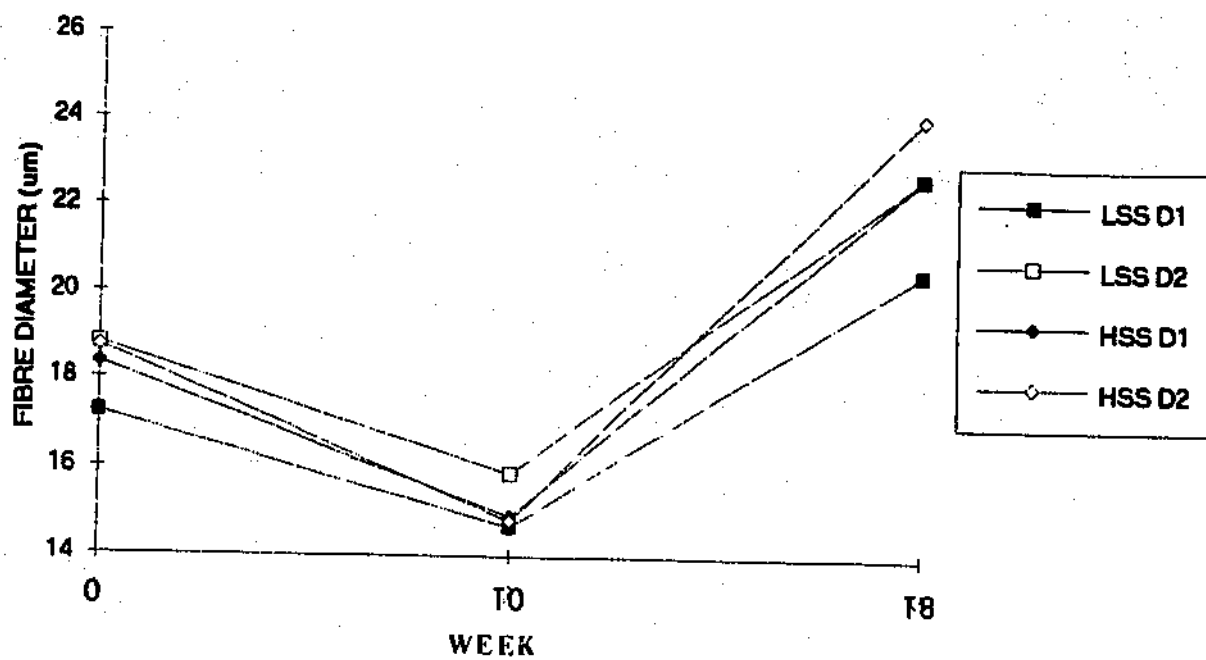
HSS-D1 = High staple strength low protein diet 1.

HSS-D2 = High staple strength high protein diet 2.

LSS-D1 = Low staple strength low protein diet 1.

LSS-D2 = Low staple strength high protein diet 2.

Figure 3.14 Fibre Diameter (um) per Group per Date



3.1.8. Multiple Regression Analysis

A multiple linear regression analysis for mitotic count (MC), follicle area (FA), live-weight (LW), clean wool weight (WW) and fibre diameter (FD) was performed to explain the relationship and statistical significance between these parameters. The resultant regression equation was:

$$WW = -3.09 + 0.0384 MC - 0.000108 FA + 0.0246 LW + 0.169 FD$$
$$r^2 = 84.5 \% \quad p = 0.000.$$

3.2. Qualitative

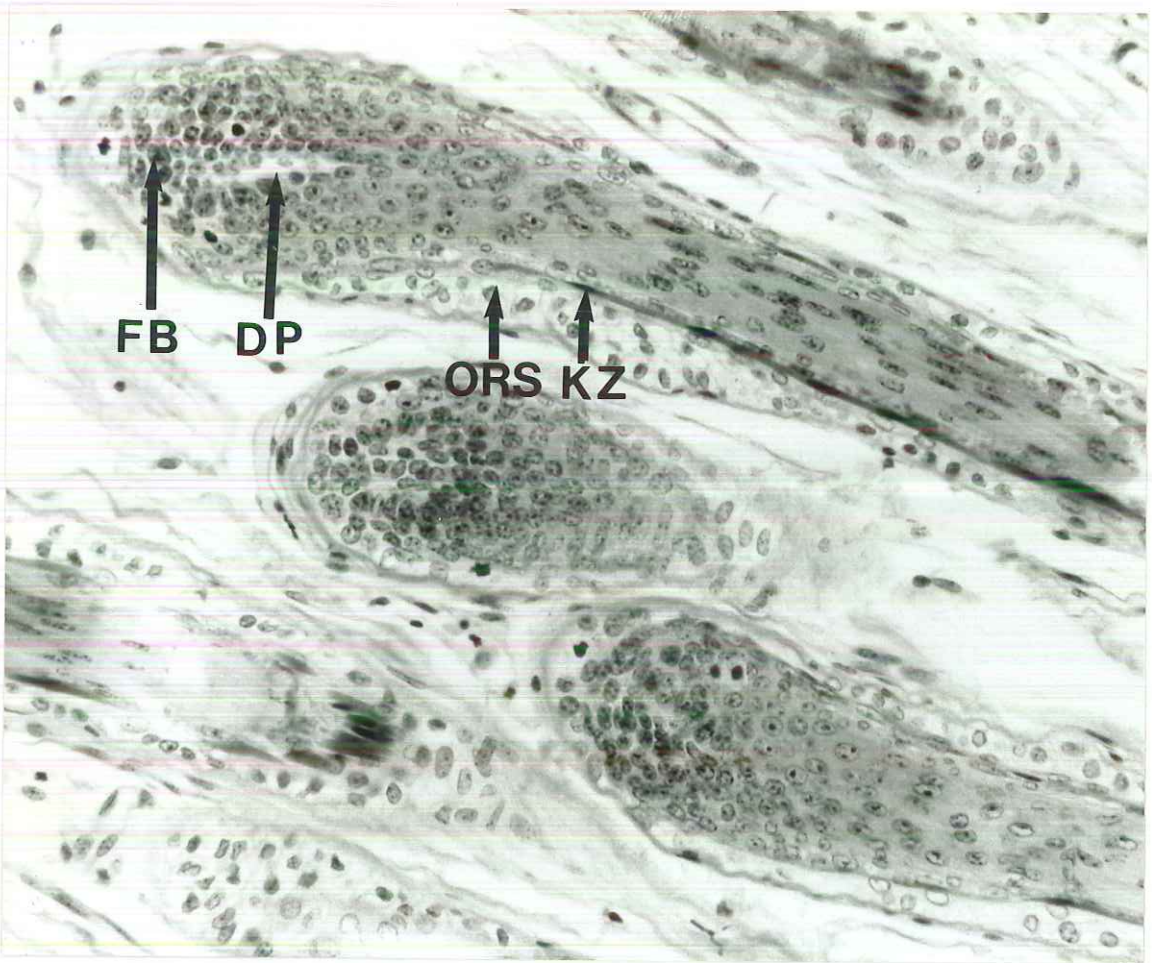
3.2.1. Stage of the Growth Cycle (Figures 3.15, 3.16)

One per cent telogen follicles were observed in fifty per cent of the sheep in each group at the time of sampling with the change of diet (that is ten weeks on restricted ration) and were not seen in the other dates (pre-treatment and eight weeks on low or high protein ration). No follicles were observed that confidently fit the catagen criteria. Each section which was examined had a number of follicles which could not be classified (8-40%) into anagen or telogen. The remainder of follicles were classified as anagen.

Figure 3.15 shows a light micrograph of a follicle in anagen. The dermal papilla can be seen enclosed in the follicle bulb. Evidence of keratinisation can be seen in the shaft of the follicle. An outer root sheath bulge is present indicating the rapid division of cells in the follicle bulb.

Figure 3.16 shows a light micrograph of a follicle in telogen. The dermal papilla can be seen as a ball of cells. The terminal end of the shed fibre, with its wool fibre, from the previous growth cycle can be seen as it migrates toward the epidermal surface.

Figure 3.15. A Wool Follicle in Anagen Stage of the Growth
Cycle



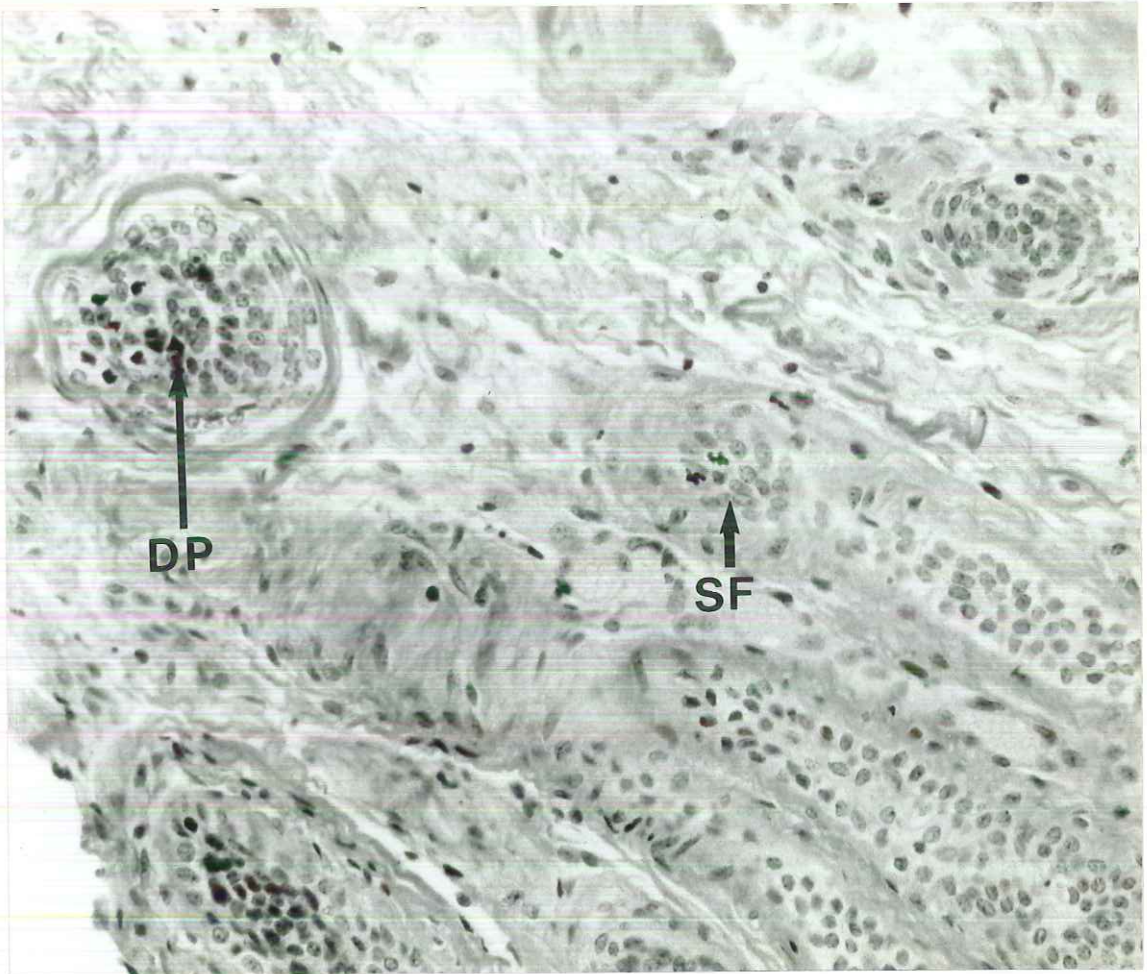
DP = Dermal Papilla

FB = Follicle Bulb

KZ = Keratogenous Zone

ORS = Outer Root Sheath bulge

Figure 3.16. A Wool Follicle in Telogen Stage of the Growth
Cycle



DP = Dermal Papilla

SF = Terminal end of Shed Fibre

DISCUSSION

The aim of this study was to investigate the effect of different nutritional regimes on the morphology of the wool follicle and its possible effect on wool production. The quantitative parameters measured were the number of mitotic cells in the follicle bulb and follicle bulb area in sheep with a history of high and low staple strength wool fibre, which were subjected to different diets over an eighteen week period. The rate of cell division and the area of the follicle bulb were related to staple strength of the wool fibre and to body weight, clean wool weight and fibre diameter, as determined by CSIRO for these sheep.

It was hypothesised that there would be a different physiological response (change in rate of cell division and area of follicle bulb) in animals to low and high protein diets in sheep with different staple strengths. This was not the case in this experiment. Each of the groups; high staple strength high protein, high staple strength low protein, low staple strength high protein and low staple strength low protein responded similarly to the nutritional regimes examined in the experiment.

Although group differences were not evident between the different diets and staple strengths, this study demonstrated a high correlation between the number of mitotically active cells in the follicular bulb, the area

of the follicular bulb, live-weight of the sheep, clean wool weight and fibre diameter ($r^2 = 84.5\%$, $p = 0.000$).

4.1. Impact of Nutritional Regime on Wool Growth

During the first ten weeks of the experiment when the sheep were offered a restricted ration; the live-weight of the animals declined by 31%. The reduction in peak mitotic rate coupled with the reduced spread of counts during this time indicates a slowing of the rate of cell division in the wool follicles. This was accompanied by a reduced follicle bulb area and is consistent with the results of Hynd (1989) who reported a positive correlation between follicle bulb area and rate of cell division. Hocking Edwards and Hynd (1992) calculated the volume of germinative tissue in the wool follicle and found it to be highly correlated with wool production as determined by clean wool weight per unit area of skin.

Fibre diameter decreased as the rate of cell division of the follicle bulb cells decreased. This reduction in fibre diameter was consistent with the findings of Harmsworth and Day (1990) who reported decreased fibre diameter in sheep with poor nutrition. Schinkel (1961) reported a correlation between fibre diameter and mitotic activity and estimated that two thirds of the increase in fibre diameter could be accounted for by an increase in cell numbers arising from the follicle bulb.

This study found that fibre diameter decreased as follicle bulb area decreased. Both Schinkel (1961) and Williams and Winston (1987) reported a positive correlation between follicle bulb size and fibre diameter.

The high correlation found between mitotic rate, follicle bulb area, live-weight, clean wool weight and fibre diameter ($r^2 = 84.5\%$, $p = 0.000$) suggests that all these parameters are effected similarly by changes in nutritional regime. There was little difference between the groups (HSS-D1, HSS-D2, LSS-D1 and LSS-D2) suggesting that the recovery from the reduced ration was similar regardless of diet or staple strength. The finding of the Kolomorogov-Smirnov test that the sheep in two of the groups (Diet 1 HSS and LSS) were statistically different to other sheep in the same group (pre-treatment) may account for some of the lack of difference in the result, however this could not be used to explain the results of the other two groups (Diet 2 HSS and LSS) which were statistically similar within groups (pre-treatment).

A field study by Butcher et al (1984) found that sheep with an initial high staple strength showed a greater decrease in staple strength than sheep with initial low staple strength, following seasonal change resulting in poorer pasture quality. This finding suggests a different physiological response to restricted rations in sheep with different staple strength and is supported by the staple

strength data recorded by CSIRO for this experiment. The high staple strength sheep fell from 34 N/ktex pre-treatment to 14 N/ktex at the end of eighteen weeks, a loss of 20 N/ktex while the low staple strength sheep fell from 17 N/ktex to 14 N/ktex, a loss of 3 N/ktex.

Hocking Edwards and Hynd (1992) found that strongwool Merinos had a greater skin uptake of glucose than finewool Merinos. There may be different factors, involved in the response of sheep to changes in diet in the field, to those involved in the response of penned sheep. Mier and Cotton (1976) and Reis (1991) indicated the importance of the ratio of amino acids in the diet. Diets containing all the essential amino acids but with unbalanced ratios resulted in reduced fibre growth. In this study the two diets had similar ratios but different amounts of amino acids, therefore the lack of difference in the different diet groups may be a reflection of this similarity in amino acid ratio. Marsten (1955) suggested that the amino acid concentration in the tissue fluids, and therefore available to cells, was determined, in part, by the quality and quantity of protein ingested in feed.

Hynd (1989) reported an increase in wool growth, as determined by clean wool weight, in sheep on a high protein diet. It was expected that in this experiment the sheep on the high protein diet would have higher wool production than those on the low protein diet. This was not the case,

in the sheep selected. There was no significant difference between the clean wool weights of the low and high protein groups. This may be a reflection of the small number of sheep used in the study or alternatively may be due to the harshness of the restricted nutritional regime causing the animals to fall below a physiological threshold for maintenance wool production. Therefore the data collected, during the eight weeks on the low or high protein diets, may represent a return to pre-treatment functioning rather than the effect of differential protein consumption. To determine the effect of differential protein consumption on sheep recovering from restricted rations it would be useful to repeat the experiment using varying degrees of nutritional deprivation before the sheep are placed onto the various protein regimes.

It was interesting to note that all of the parameters measured returned to a level higher than pre-treatment after eight weeks on the low or high protein diets. This was possibly due to the nature of the diet being higher in energy than the pre-treatment diet. Moore et al (1982) also reported an increase in wool production in sheep fed a diet higher in energy but similar in content. The higher than pre-treatment levels found in this study may reflect an over-compensation, by the sheep, for the losses suffered during the time on the reduced diet.

This is the first time that telogen has been described in the Merino. The limitations of planar sections has made these descriptions superficial and three dimensional reconstruction would need to be carried out to describe accurately each sub-stage of catagen and telogen stages of the growth cycle in Merinos. Extensive work needs to be undertaken to describe the morphology of the telogen follicle in the Merino. The absence of follicles in the telogen stage of the growth cycle in the pre-treatment and low or high protein ration groups suggests that descriptions of telogen follicles would need to be undertaken for sections from animals on restricted rations to maximise the potential of identification of the stages of the growth cycle.

Ryder (1973) suggested that in sheep a mechanism involving more than one follicle is involved in stimulating a new wool growth cycle. If this is the case it could be expected that all follicles within a given area would be in the same stage of the growth cycle. However in this experiment the sheep in which the telogen follicles were found had only one per cent of follicles in telogen. This finding suggests that the shutdown of follicles may be independent of the stage of the surrounding follicles. It is possible that sheep on a restricted ration for longer than ten weeks would show a more pronounced shift towards telogen. It is possible that the low number of telogen follicles observed in this study is due to the fact that the samples represent

a single point in time. A time series would give a better indication of the number of follicles in telogen. It is also possible that the low percentage of telogen follicles found in this experiment represents an initiation of follicle shutdown which may spread to neighbouring follicles with time.

The observation that telogen follicles were found only in the group that had consumed the restricted ration would suggest that undernourishment was causing a shift to telogen or follicle shutdown. The reason some follicles shutdown under poor nutrition is not known but it may be the result of preferential supply of nutrients to selected follicles at the expense of others. A shift to telogen accompanying restricted nutritional intake was also reported in human children suffering from the deficiency disease Marasmus (Bradfield and Bailey 1967).

Chapman and Basset (1970) found that sheep on restricted rations were more sensitive to changes in plasma cortisol. These sheep shed wool fibres (indicating that wool follicles were entering the catagen stage of the growth cycle), when their plasma cortisol levels were raised. As the adrenal cortex has been shown to increase in activity during stress, it may be that a rise in plasma cortisol, at the end of ten weeks on restricted rations, influences the sheep's wool follicles to enter telogen.

4.2. Model for Wool Growth

Black and Reis (1979) proposed the following model for wool growth; that the amount of wool grown depends on the number of wool follicles, which is under genetic control and can only be modified during foetal development. Each animal therefore has a potential to develop a maximum number of wool follicles at birth. The fibre production of an individual follicle is determined by the number and size of cells in the proliferative region of the bulb, the rate of division of bulb cells and the proportion of migrating cells that enter the fibre. Most of these factors are considered to be genetically determined with the major environmental influence being nutrition being which would affect the rate of cell division in the follicle bulb. Alterations in the hormonal or physiological status of an animal would cause changes in affinities for substrates of metabolic reactions in tissues other than wool, thereby altering amounts of nutrients available to the follicle, resulting in changes in the rate of cell division. Wilson and Short (1979) calculated that cell number and mitotic activity together accounted for ninety per cent of the variance in fibre production between sheep. As rate of cell division appears to be directly related to follicle bulb area selection of sheep for breeding based on bulb area may increase wool production.

Scobie and Woods (1992) suggested that follicle volume was the major determinant of wool growth and the results of this study tend to support this. This study found that follicle bulb area was related to mitotically active cells of the bulb. If follicle volume could be proved to be the major determinant of wool production then sheep could be selected on the basis of follicle area at an early age as an alternative to the current practice of double shearing to estimate wool production.

4.3. Future Directions

When the nature of the impact of diet on staple strength is more fully understood then the production of sound wool may be possible and would have major economic gains for the wool industry. Further work is indicated in the relationship between staple strength, diet and wool production. Wynn et al (1991) stated that current evidence suggested that differences in wool production in individual sheep were due to differences in follicle function which may be determined genetically. Therefore when follicle function is more fully understood, selection for the characteristics reflecting follicle function which are involved in producing less tender wool may become possible.

Three dimensional reconstruction is required to provide accurate descriptions of the various stages of the growth cycle of the wool follicle in sheep. Few descriptions of

wool follicles during the various stages of the growth cycle are available and at this stage much of the work has been done on other species, particularly the mouse and rat. Three dimensional work would also provide a useful measurement of follicle volume which may be an indicator of potential wool production when follicle density is taken into account.

Roth (1965) and Parakkal (1990) have studied mouse hair follicles in the different stages of the growth cycle at electron microscope level. At present little is known of the ultrastructure of sheep follicles in catagen and telogen and it would be useful to expand the knowledge of the morphology of the wool follicle during the different stages of the growth cycle at light and electron microscope level.

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